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Introduction

Good management and a fertile stallion can lead to success in a breeding program. But when problems arise with either of these factors, early veterinary evaluation and intervention can influence the future success of that operation. These papers offer valuable information on not only how to avoid problems but also how to readily detect abnormalities and deal with these issues should they occur. Time and patience are well spent when starting to breed a novice stallion. Clear handling and well-planned routines can have a lifelong positive influence on a stallion's breeding behavior.

The initial use of transported semen offered tremendous opportunity to breed horses that may otherwise have been geographically inaccessible and allowed both performance stallions and mares to remain at their training facilities. This management tool has become more popular than was ever expected and is even being offered by more traditional breeding farm operations. But as with so many assisted reproductive techniques, the use of transported semen requires exceptional attention to many details or fertility may decline. Success in a transported semen breeding program requires superb breeding management of the stallion and mares, effective communication between all parties involved, and excellent semen handling techniques. The paper on breeding mares with transported semen provides recommendations for the successful use of these techniques.

The equine industry in the United States is tolerant of variable semen quality in breeding horses as desirable sires are selected for performance traits or conformation characteristics rather than innate fertility. Much is known about sperm morphology in food animals, and the etiology of many of their sperm abnormalities are understood. Although it is known that morphology is not the definitive predictor of stallion fertility, it is commonly assessed during stallion reproductive evaluations. This issue contains what may be the first comprehensive effort at reviewing sperm morphology in stallions.

Ultrasonography of the stallion's genital tract is considered a routine part of a breeding soundness examination and can allow for an accurate measurement of the testicles. The paper that describes the evaluation of testicular vasculature also reviews the importance of blood flow to the testes. Testicular vasculature evaluation can be useful in investigating many of the conditions associated with an enlarged scrotum. The diagnosis and management of these conditions are presented. Unfortunately, some of these scrotal problems eventually lead to testicular degeneration. But in many cases, a clear etiology of testicular degeneration is not understood. The paper on testicular degeneration will help the reader understand the pathogenesis of, and more readily diagnose and manage, this problem in stallions.

The risk of infectious disease in breeding stallions is great in our global world. The review of infectious diseases in stallions summarizes the problems that our stallions face when breeding mares from many different locations. We must be knowledgeable of the clinical signs of these infectious diseases and quick to respond to any outbreaks should they occur. This issue concludes with some thoughts and advice from an experienced theriogenologist who has spent years working in the Thoroughbred breeding industry in central Kentucky.

Patricia L. Sertich, MS, VMD
Guest Editor

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Starting a Novice Breeding Stallion

Sue M. McDonnell, PhD

The manner in which a stallion is initially introduced to breeding can have long-lasting effects on his breeding behavior, with implications for safety, and breeding efficiency of the horse. So successfully starting a novice stallion in an organized positive manner has become a typically satisfying challenge for our team. This how-to paper details what we at our veterinary school stallion referral clinic believe to be key handling concepts, facilities, equipment, personnel, training schedule and goals for getting novice breeders off to a good start. Also included is the specific breeding shed protocol that is used routinely at our stallion referral clinic for natural service or semen collection, as an example of one method that has worked well with all types of stallions in a teaching environment that involves new student handlers with a variety of levels of skill and experience.
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Starting a novice breeding stallion can range from a quick and easy project accomplished in a few brief sessions to a challenging and time-consuming effort over many sessions. Examples of specific challenges include the slow responding stallion, the sexually inhibited or "confused" stallion, and the over enthusiastic yet awkward novice. With experience, our team at our university referral practice has come to enjoy working with even the most challenging beginners. Every stallion teaches us something new or reminds of the various procedures to try to speed things along. We have come to appreciate that how the challenges of the novice breeder are handled can significantly impact the stallion's breeding success. This paper will summarize our general approach and specific procedures as well as practical tips for efficiently starting breeding stallions.

Getting Organized

Our approach to facilities and personnel for breeding and semen collection certainly falls on the minimalist end of the scale. "Simpler" really seems better for us in most instances. We have only a few basic recommendations for facilities, personnel, stimulus and mount mares, equipment, and supplies for starting a novice stallion.

Equine Behavior Clinic, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, Kennett Square, PA.
Earlier versions of this manuscript were prepared for the 2004 Bluegrass Symposium and for The 2006 Kentucky Veterinary Medical Association Annual Meeting.
Address reprint requests to Sue M. McDonnell, Equine Behavior Clinic, University of Pennsylvania School of Veterinary Medicine, New Bolton Center, 382 West Street Road, Kennett Square, PA 19348. E-mail: suemcd@vet.upenn.edu

Facilities

Breeding or semen collection can be done efficiently and safely in a variety of types of spaces. In general, we prefer a large clutter-free space, especially for training a novice breeder. We recommend that the shortest dimension of the breeding area be at least 25 feet. Our stallion handlers find an area of roughly 40 feet × 40 feet workable for even the most enthusiastic stallions. In general, it is our opinion that, the smaller the space, the greater the skill and organization required to safely avoid accidents. We recommend that the area be enclosed to reduce complications should an animal get loose from the handler. A purpose-designed and dedicated breeding shed may be ideal for larger operations, but a grass paddock, a wide barn aisle, or an arena are workable alternatives. If the area is indoors, we like wide doorways (at least 10 feet wide) on at least two sides of the room for easy exit with animals if necessary. Good footing is a high priority, especially when starting new stallions. It is best to have a surface that is not slippery, even when wet. We prefer a footing of solid rather than loose material. This is because loose material invariably gets kicked up onto the penis where it can be abrasive and get into semen or the mare reproductive tract. It is best if the area selected for breeding is quiet and out of the busy traffic area. Some novice breeding stallions are easily distracted. If the area is indoors, ample headroom, with at least 14- to 16-foot clearance to the lowest fixture, is recommended. This will accommodate the largest stallions should they rear and extend their forelimbs. A teasing stocks, wall, or rail in the breeding area is also useful, but not necessary.

Some of the common facilities pitfalls to avoid include any number of things that seem ordinary to people, but can be off-putting at just the wrong moment to an anxious novice breeding stallion. Examples include windows that cast light

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or reflect the animal images or movement, floor patterns or irregularities such as drains or wet spots, and sources of noises such as fans and other motors that cycle on and off.

Personnel

As with facilities, the type and expertise of handling teams vary widely throughout the equine breeding industry. We prefer a small team over a larger crew, especially when starting a novice. For natural cover, this includes a stallion handler, a mare handler, and one assistant available if needed. For semen collection, this includes a stallion handler, a mare handler for the stimulus and/or mount mare, and a semen collection technician. For some novices, a common challenge is to keep the stallion squared up at the rear rather than progressing up the side toward the head or over the barrel of the mare or dummy mount. In such cases, we find it useful to have an assistant available to "spot" at the hip of the stallion. Most stallions that thrust vigorously tend to "travel" up the near side if not encouraged to remain squared up at the rear of the dummy mount. In rare instances, a disorganized beginner will also tend to scramble up on the off side of the dummy mount or mare. In that case, an assistant on each side of the stallion ready to "spot" if necessary can be helpful in getting the stallion squared up for insertion and organized thrusting. In our experience, for all but the most aggressive and strong stallions, these spotters are needed for only a couple to a few initial sessions before the horse settles down or learns to couple squarely behind the mount without assistance or with just the nudge of the semen collection technician.

At our teaching facility, the make-up and experience of the team vary from day-to-day. We find it important before we start a session for each member of the team to understand (1) the general goals and plan for the particular horse, (2) the particular goals for that session, as well as (3) the specific role for each of the team members participating in session. Before a session begins, we also review the anticipated challenges and the contingency plan for safe resolution, eg, should a crisis arise, where the stallion and handler will go with the stallion, where the mare and handler will go with the mare, and who will make the "call" and direct the team should a crisis arise.

Personal safety gear used for breeding horses varies considerably among facilities. Experienced handlers, if not accustomed to wearing safety gear with horses, may judge that the gear encumbers their agility and perception in the breeding situation. In my experience, teams that routinely use personal safety attire quickly acclimate and judge that the safety equipment adds security that imparts additional confidence. At our facility, we have safety vests and helmets available. This gear is used on a case-by-case basis and is the personal decision of each team member. Safety shoes that are specifically rated for steel industry or equine activities are used routinely by some individuals. Others prefer lighter athletic shoes and take the strategy of avoiding being stepped on.

To reduce distractions, both for people and animals, we encourage team members to turn off or silence mobile communication devices during stallion training and handling sessions.

Figure 1 Leather stallion breeding halter (Quillin Leather and Tack, Paris, KY; http://quillin.com/jshop/product.php?xProd = 967&xSec = 21&jssCart = 58cfbe4f36c4f57a2e5d1d60113c2434. Also available from Pinkston's Turf Goods, Lexington, KY; http://www.pinkstons.com/halters.html.). (Color version of figure is available online.)

Stallion Restraint

There are many types and configurations of halters and leads that can be used effectively for handling stallions for breeding. Essential to effective use of any of the configurations is that the handler understands the particular features and limitations. At our facility, by custom as well as with experience teaching primarily novice or unskilled handlers, we generally use a very simple halter and chain shank configuration. The lead is an 8-foot cotton shank with a 40-inch English brass chain. Ideally, we use a purpose-designed leather breeding halter that is well fitted to the horse (Fig. 1). A stout nylon halter with good brass fittings is an acceptable alternative. The leather halters designed specifically for breeding stallions have 1.25-inch double thickness supple triple-stitched nose band, cheek, and crown pieces. The throat latch is half-inch rolled leather shaped to the contour of the jaw. One-inch quarter-circle leather stays connecting the nose band to cheek pieces prevent rolling and rotation. For balanced directional control of the stallion's head, the nose band should be adjusted to sit approximately mid-way down the bridge of the nose, well above the crease of the lip and with approximately three fingers of slack. Another important feature of a breeding halter, especially when using a chain shank configuration, is high-quality brass fittings that are rounded in all dimensions.

The purpose-designed breeding stallion halters usually have 4-gauge, 2-inch brass rings. When putting the chain on and off, the snap end will pass easily through the 2-inch diameter ring, and the snap is easily attached and removed from the 4-gauge rounded stock. When handling the stallion, a similar high-quality soft brass oval-link chain glides smoothly through the cheek rings. Fine increments of tension can be smoothly applied and released to guide the stallion. Especially important is the instantaneous and smooth release of pressure to return to light touch as a reward for the desired response. In contrast, rings of poor-quality metal or flat stock or square-cornered, or poor quality chain, result in

unavoidable prolonged and/or heavy injudicious pressure. Even in skilled hands, this makes use of a chain shank inefficient for basic respectful control and behavior modification. With a good fit, these stout breeding halters appear to impart a positive level of "psychological" control, not too loose or too confined.

Depending on the history and temperament of the stallion, the skill and preference of the handler, and any specific recommendations by the owner or trainer, we will begin with the chain either over the nose or through the mouth over the tongue. In either configuration, the chain is put through the near and off-side rings and then up to attach to the high off-side ring. This high off-side attachment of the shank gives most handlers smoother and lighter directional control of the stallion. With extremely shy stallions, we may simply clip a cotton lead to the lower ring on a halter. Use of the chain over the nose, and especially use of a chain over the tongue, requires some training and skill to be effective and humane. It takes some skill to maintain slack on the chain, and to apply measured tension only as needed to cue and guide the stallion. If tension is continuously applied, or if injudicious tension is applied or inadvertently caused by failure to move with the horse, the method becomes ineffective and counterproductive. The goal is not to jerk the chain, or to apply punitive pressure, but rather to provide gentle guiding tension similar to the use of a bit in riding or driving. Specifically, jerking of the chain should not be used as a form of "attention getting" or punishment. Whereas many horses can learn to tolerate this misapplication of a chain, for some the jerking of the chain on the tongue or nose may insight dangerous evasive or aggressive reaction. This can include striking, rearing, or even boxing while rearing as if fighting the handler or the restraint. Horses can get used to being jerked around or to the nervous jerking on a chain, and some not only tolerate it but appear to become conditioned to it as a predictor of breeding opportunity. So some handlers mistakenly conclude that this is an appropriate or even necessary handling style. Alternatively, "jerking a stallion's" restraint can lead to "learned helplessness," known to horseman as "souring" or "sulking," which is clearly counterproductive.

Figure 2 Hands-free gum pressure restraint. Stableizer® available from Valleyvet.com. http://www.valleyvet.com/ct_detail.html?PGGUID = 2e87c082 to 7b6a-11d5-a192 to 00b0d0204ae5. (Color version of figure is available online.)

Other configurations of the chain under the chin or completely around the nose or diagonally over the nose or under the chin can be used effectively.

A more severe level of restraint is a gum chain. In skilled hands, a gum chain can effectively calm some over enthusiastic stallions in the breeding situation, especially those that tend to rear. However, even more with the gum chain than with the chain over the nose or tongue, just the right level tension must be maintained so as not to exacerbate those tendencies, provoke even savage aggression, or injure and sour the stallion to breeding. Our experience has been that a much higher level of skill is required to effectively or humanely apply a gum chain for breeding than other chain arrangements. A handling device designed to apply steady pressure to the gum is commercially available under the brand name Stableizer® (Fig. 2). It is the author's experience that considerable skill is required to appropriately apply such a device for use in breeding situations.

Breeding bridles that include bits, although not as common in North America, are customary regionally around the world. It has been my experience that a very high level of skill is required to effectively and judiciously use a breeding bridle without inadvertently inciting problematic behavior. With this type of restraint, there seems to be an unusually high incidence of rearing and striking that seems provoked by the bit restraint. In evaluating problem situations, as well as observing handlers skilled at using a breeding bridle, it appears that certain stallions behave as if "trapped" and thwarted by the stout bridle and bit arrangement. Their behavior suggests a conspicuous approach-avoidance anxiety state, with some continually "on the edge" of exploding or attacking. This seems to reduce the tolerance of the stallion for inadvertent or nervous jerking on the bit by the handler. With time, some stallions show signs of a "learned helplessness," loss of libido, or even an aggressive or passive souring. These behavioral changes can result from misapplication of any head restraint or pattern of repeated injudicious correction in the breeding situation, but seem more common with use of a breeding bridle.

Although we would not recommend the practice, we have known of stallions for which a nose twitch was used for control during breeding. As with using a twitch for mares in the breeding situation, particular skills must be required to safely manage a twitch in addition to directing the stallion. As with a mare, the stallion should not be led (pulled or pushed) around by the twitch, but rather directed by a primary halter and lead.

Stimulus and Mount Mares and Restraint

Ideally, for novice stallions, it is often useful to have a variety of mares available. For the enthusiastic stallion for semen collection, a mare may not be needed at all, and progress may in fact be better without a mare present. For the slow starting or lower libido stallions, it is often useful to have two or more naturally cycling mares in good natural estrus. Some stallions, especially novice breeders, seem to show preferences, and also tend to tire of one mare. A good ovariectomized or anestrous (given estrogen if needed) mare can be adequately stimulating for most stallions, but some are clearly more responsive to naturally cycling mares. We like to have alterna-

tive mares nearby and all ready for use when we start working with a stallion so that options are readily available. Restraint for the stimulus mare should be the "minimum safe level." In fact, for starting a slow novice, it is often best to limit the restraint of the stimulus mare so that she can express natural behavior.

For starting a stallion to in-hand natural service, we recommend a reliably tolerant mount mare of an appropriate size. If an ideal size match is not available, too tall is often better than too short. Stallions tend to "belly out" and paddle up the side of short mares. We routinely use an ovariectomized mare with minimal restraint that has proven trustworthy with awkward or rowdy stallions. We typically use a cotton lead and a twitch to restrain these mares for mounting. In some cases, a mare will appear to be more stimulating if not twitched, and we have mares that have proven to be safe without a twitch that we will use with only a cotton lead. We also routinely bag the tail and apply lubricating jelly to the vulva, but if these steps appear distracting or off-putting to a novice, we eliminate them.

If our ovariectomized mares are not sufficiently stimulating to a novice, we switch to a natural estrous mare. If we are certain that the natural estrous mare will be a "safe" mount, we may tease the stallion to the natural estrous mare and then divert to a nearby ovariectomized mount mare that is trustworthy for a novice. Most novices exhibit variable response among mares, so we try to have a selection of natural estrous mares readily available.

Penis Preparation

We use clean warm water (115°F) and a cup for pouring water to remove loose debris, and lint-free disposable cotton towels for blotting dry and assessing any remaining dirty areas. Trash can liners work well as a clean disposable bucket liner.

Semen Collection

For starting a novice to semen collection, we typically try the stallion on the dummy mount from the start, but also have no hesitation to start with a mount mare for initial sessions and then advance to the dummy mount. For dummy training we usually try first without the mare near the dummy, since many stallions will respond to a dummy mount with minimal stimulation. If not, we then put the stimulus mare next to the dummy. Or we may allow the stallion to follow a step or two behind the mount mare as we approach the dummy, carefully guiding the stallion at the last moment to the dummy as the mare is led aside. For training to semen collection, we tend to use a fairly warm and full AV with ample lube to provide most inviting stimulation. We also have hot compresses ready to apply to the base of the penis for added encouragement to thrust.

The dummy mount position, height, and design features can greatly facilitate or hinder training efficiency in a novice breeder. Detailed recommendations for design and fit of dummy mounts have been discussed in detail in an earlier publication.[1]

Other Items

When preparing to work with a novice we also have a few additional items handy should we wish to try them. These include:

- Half- and full-cup blinker/blinders can be useful in a number of instances. They can improve "focus" for distracted stallions. These can also be useful for facilitating the "bait and switch" procedure described above. Also, some rowdy stallions are more manageable with blinkers or blinders.
- Estrous mare urine (can be kept frozen) can be useful for improving interest in a dummy mount or a stimulus or mount mare.
- Knee wraps, topical anesthetic, and emollient ointment can be helpful for protecting from or soothing carpal rub sores that are a common problem in beginner breeding stallions, especially with dummy mounts.

Getting Started

Although we customize the training protocol and schedule for each novice stallion, the following 10 steps summarize how we proceed with training.

Step 1: Establish or Refresh General Handling Rapport with the Stallion

Before entering the breeding situation, we find it useful for the stallion handler to work with the stallion, using the restraint that will be used in the breeding situation. Ideally this is done for a few days before the start of training. But realistically, at most clinics it is done on the day the stallion arrives for training or for the first breeding or semen collection. Before beginning the breeding training session, even just a few minutes of walking the stallion in a nonsexual situation using the restraint that will be used for breeding can be helpful in establishing clear communication with the stallion. Specifically we recommend to establish gesture and verbal commands for the following basics—walk, stop, stand, and back. For most previously schooled horses, even only three or four replicates of these commands will give the new horse and handler team confidence that will be invaluable in the breeding situation. Regardless of the history or temperament of the horse, we feel this step is worthwhile. A further useful step is to similarly handle the stallion in the breeding area before a mare is introduced. This will familiarize the stallion with the area and reinforce your basic commands in that area.

Step 2: Establish Rapport with the Stallion in a Teasing Situation

Before first entering the breeding area, we also like to assess the behavior of the stallion in the presence of mares and to again reinforce basic handling communication, again using the halter and lead that will be used in the breeding situation. The same basic ground commands can be used to convey to the horse that, with the direction of the handler, he can approach and interact with the mare in an organized manner.

Step 3: Entering the Breeding Shed

Our first goal when entering the breeding shed is to have the stallion enter the area and to become oriented to the stimulus mare from a distance of 20 to 30 feet at first. Probably half of novice breeders will achieve and maintain an erection for preparation of the penis within a minute or so when paused at this distance, and so will not need to tease up close to a mare before penis preparation. If not, we then proceed closer to the mare as needed. As we gradually approach the stimulus mare, the handler's goal is to convey to the stallion that he can approach the mare at the pace of the handler. If he rushes ahead, as novices often do (sometimes unexpectedly in fits and spurts of interest), we recommend avoiding punishment or catastrophe. If needed, we recommend backing the stallion as calmly as possible away from the mare or even retreating from the breeding area and regrouping for a more organized approach. The goal is to be as organized and positive as possible. Circling the stallion is specifically discouraged. This is because stallions at liberty have a natural tendency to circle away from a resistant mare, gesturing a kick threat as they circle. As with the strike threat, this is not usually a safety issue for the mare or stallion at liberty. In confined spaces, this kicking out while circling can be deadly. We try never to circle a stallion near a mare in a confined breeding situation; rather we back him away using the gesture and verbal command established earlier.

Step 4: Close Teasing as Needed

Many novice breeders will not respond sufficiently until allowed closer contact with the stimulus mare, at least at first. Approaching head-to-head, and commencing teasing from the shoulder toward the tail is the natural sequence for horses breeding at liberty. The natural sequence also includes strike threat gestures when they are approaching head-to-head. Whereas a strike threat is not a safety issue for animals breeding at liberty, in a confined breeding situation, the strike threat can be dangerous if not anticipated and carefully directed. The sequence also includes squealing and barking vocalizations, that can be unsettling to the unsuspecting handler. For this reason, many handlers direct initial teasing to the rear of the mare. This works adequately for many stallions. But most respond much more enthusiastically when allowed to first approach head-to-head. Turning the head of the mare back toward the stallion can simulate the mare's natural behavior and can induce sudden arousal for most stallions. Some beginners will not respond adequately, even after long periods of exposure to a mare that is confined in stocks. Moving the mare out into an open space, and directing the mare to move a step or two at a time, or forward and back slightly, or allowing the mare to interact and posture more naturally is often needed to stimulate a novice stallion.

Step 5: Penis Preparation

We proceed with each stallion with the plan of introducing manipulation and preparation of the penis from the start. But if the horse is especially shy or resentful of penis manipulation, or has fairly low arousal, we may delay the procedure until subsequent sessions when the horse is judged ready. We view this as a simple behavior modification procedure similar to picking up feet or tolerating another noninvasive procedure that all horses can learn to abide with positive reinforcement. With the stallion handler on the near side of the horse, the wash technician approaches the stallion at the shoulder, and then moves the back of the left hand along the abdomen and then firmly, but gently grasps the shaft of the penis (Fig. 3). Our goal is to maintain contact of the left side of the technician to the shoulder or abdomen of the stallion, so that we can more safely "ride out" minor resentment or evasive maneuvers of the horse. Having to bail out repeatedly can put the horse in what is called in behavior terms an "avoidance cycle," that can quickly accelerate into dangerous maneuvers. At the same time that we commit to staying with

Figure 3 Washing and drying the stallion's penis. (Color version of figure is available online.)

the horse, we try to progress to pouring warm water on the penis as soon as possible. For most horses the warm water appears to be immediately positive and helps them to tolerate further manipulation. Important aspects of washing include a confident, reassuring approach and gentle handling of the delicate tissues. Some tips to gain and maintain compliance of the stallion with this handling and washing the penis are:

- For a stallion that is reactive to approach or manipulation of the penis, it can be helpful to begin by stroking the belly starting at the shoulder and gradually moving toward the penis. The stallion handler can sometimes more safely do this before the washing technician approaches.
- Gentle massage and blotting as opposed rubbing and scrubbing motions are more readily tolerated.
- Avoid splashing the hind legs with water, which for some horses seems off-putting.
- The wash can be abbreviated or eliminated for the first session, or until the horse has had one or more successful ejaculations, but then increase the time spent each session devoted to washing until the horse is routinely tolerating a full examination and wash before mounting.
- For horses that are learning to tolerate the washing, but have not yet succeeded, try to end each session on a positive note. Continue until a moment of calm tolerance as opposed to ending the attempt amid a moment of resistance.

Step 6: Re-Teasing and Mount

Once the stallion's penis has been washed, we recommend moving the stallion to a distance from the stimulus mare, where the stallion and handler can stand calmly while last minute preparations of the mare or artificial vagina are completed. When all is prepared and the team is in place, the stallion is re-teased if necessary in a manner similar to the initial teasing. Once the stallion has a fully rigid erection and is ready to mount, he is led up to the mare or dummy mount at a slight angle on the near side and encouraged to pause momentarily and then mount when directed by the handler (rather than to leap from a distance or rush ahead of the handler's direction). Some stallions naturally tend to mount from the side and then rotate around to the rear position. This is what is done in the natural environment. This can be safely allowed under most circumstances. For natural service, when hobbles and other restraint items are employed, the sideways mounts can lead to the stallion getting entangled in the gear or injuring the mare handler(s), and so many facilities prefer and enforce the rear approach. Most stallions can learn to trust the handler to bring them safely from the rear, but at first this can significantly delay training and confuse some novices. We try to safely abide it at first and then work toward a rear mount later.

Some stallions seem to stall at the point of mounting, as if they don't know what to do next, or are afraid of mounting. Stimulation of the chest, by bumping into the mare or dummy, can elicit mounting. For stallions that stall, teasing to a higher level of arousal before approach, or allowing the stallion to tease close up, especially at the head, will typically move things along. Walking a mare a step at a time in front of a stallion with jerky stops can be both stimulating mentally, and accomplish a chest bump to physically stimulate mounting. If the goal is to mount a dummy, the mare can be led up alongside the dummy, and when ready to mount the stallion can be diverted to the dummy.

Many novice stallions lose their erection on approach of the mare and try to mount without an erection. This is a natural normal step in breeding under natural conditions. This behavior appears to be a final testing of the mare's readiness, before risking injury of the penis. Allowing mounting without erection usually moves things along more efficiently with a novice than enforcing erection before mounting. This tendency is typically lost once the stallion gains the trust of the breeding situation and the handler to "make the safety decisions," so to speak.

Also, stimulation of the penis, either with a well-lubricated, warm artificial vagina, or with a very warm compress against the glans penis, can elicit thrusting and mounting. Touching of the penis in a novice can also incite resentment, so care is taken to stand safely away from the hind legs. For clinicians who are new to this procedure, we find it helpful to first practice with a trusted stallion, to learn how to position yourself for greatest safety should the stallion kick or "explode," and to gain an appreciation of how to move up with the stallion as he mounts with the artificial vagina in place.

Step 7: Insertion and Thrusting

For natural service, facilities vary on their degree of assistance provided to the stallion with insertion. Most stallions and mares in estrus can safely accomplish insertion without assistance, and most stallions can learn to tolerate assistance. When collecting semen using a dummy mount, to prevent injury of the penis, care should be taken to immediately direct the penis. This should be done whether the artificial vagina is built into the dummy or hand held. Most horses will thrust on their own, but some novices seem to stall or "hang" without fully coupling and thrusting. In such cases, stimulating the penis with a warm compress or artificial vagina can draw the horse up. Sometimes several mounts are needed to accomplish good rhythmic thrusts. This pattern can also occur with horses with musculoskeletal discomfort and neurologic problems, so it may be difficult to sort out at first.

Figure 4 Novice stallion lingering on the dummy mount after ejaculation. (Color version of figure is available online.)

Step 8: Dismounting

Whenever possible, it is usually best to allow the stallion to remain undisturbed for several seconds after ejaculation to dismount when ready. Under natural conditions, the mare plays an active role in enabling dismount, by stepping forward. So, many stallions must learn how to dismount gracefully from a dummy mount. Novices may linger on the mare or dummy mount after ejaculation as if confused about what to do next (Fig. 4). Occasionally, novices will appear to faint with ejaculation, and so need to be stimulated, for example with a slap to the shoulder, so as not to fall during dismount. When dismount commences, a mare handler can facilitate dismounting by stepping the mare forward as the stallion stirs to dismount.

Especially for mounts during unsuccessful mounts, but occasionally after successful breeding, some stallions have the tendency to turn and kick out toward the mare or dummy mount as they dismount. This turning and kicking is seen in teasing and mating under natural social conditions, where it appears to be a threat gesture similar to that seen in other thwarted goal situations. Under natural conditions where there is plenty of space, the kick rarely makes contact. Under hand-breeding in confined spaces, this behavior can be dangerous to handlers, the mare, and unnecessarily to the stallion. The handler can usually avert the gesture by remaining alert to the possibility and directing the stallion back a few steps before turning the stallion.

Step 9: Rest and Reinforcement

We try to reinforce improvement, whether or not ejaculation occurred, with a pat on the shoulder and positive tone of voice, and then allow the horse to stand and rest in the breeding area for a minute or two after ejaculation.

Step 10: Leaving the Breeding Area

Some stallions are reluctant to leave the breeding area and the mare. Sometimes this appears to be because they have not yet "gathered their wits." Under natural social organization, the harem stallion "tends" the mare, remaining nearby to protect her from harassment by other stallions. So for stallions that are reluctant to leave, we just wait a minute or two, remove the mare from the shed, and then encourage the stallion to leave without a battle. Occasionally, we have a stallion that does much better if allowed to follow the mare back to his stall. We usually decide to abide that rather than to battle with the stallion. Many people establish a routine of a cool bath or pasture turn-out immediately after breeding. This can be a great positive reinforcement for a good job and for leaving the breeding area. Reluctance to leave the breeding shed typically wanes with experience with your routine. Occasionally, we find a stallion that retains this tendency through a long breeding career.

Polishing the Teamwork and Reinforcing the Routine

Initially, we try to be as patient and tolerant as safely possible of the beginner stallion. This is whether the stallion's behavior is slow and confused, or enthusiastic and sloppy. Once ejaculation has occurred, most stallions appear to have ample motivation and confidence to abide necessary direction and procedures. With each subsequent session, our goal is to provide consistent handling, so that the horse can efficiently learn our routine. We also try to plan for and appreciate improvement with each session, rather than to demand immediate perfection. In our planning for subsequent sessions, we review the specific progress and areas for improvement for the stallion.

It is our impression, that for the enthusiastic stallion, training is most efficient when sessions are repeated over a short period of time. So for an enthusiastic novice, we generally recommend working three or four sessions per day for a few consecutive days. After a break of even of a few days, it is often our impression that the stallion's enthusiasm is too great for organized learning sessions.

For stallions with low libido, once or twice daily sessions may be best at first. If libido diminishes, one session every other day can sometimes be a more efficient training schedule. For slow stallions, we tend to work in half-hour sessions, or even shorter if interest diminishes or frustration of the horse or team is apparent. We try to end on a good note. So if there is an explosive moment, for example, should the horse rush ahead of the handler, strike, or rear, we try to continue on a minute or two to end the session at a more positive point while the horse is doing well.

We generally request that the stallion be available for a few days for training for breeding. We prefer that the stallion stay at our facility so that we can work at times and on a schedule that are most productive for the horse and for the people. In reality, however, probably the majority of the stallions we start are brought in for brief sporadic training. For some stallions, that works satisfactorily. For others, it is our impression that organized breeding or semen collection is clearly delayed and not as time- or cost-efficient as a few days' stay dedicated to training.

Once a stallion is comfortable with the general handling and routine, an additional step in his training is to gradually introduce some variety, so that the stallion can experience and accept some change of routine. This avoids creating what can appear to be a ritual-bound breeder. Changes would include locations of breeding, handlers and assistants of varying levels of expertise, and the specific order of protocol. This step also helps us better understand and address potential challenges that may arise for the stallion with various handlers and conditions.

Additional Resources

Breeding shed personnel may benefit from an understanding of natural horse breeding behavior[2] and the implications of behavior modification necessary for safe handling under domestic breeding conditions.[3,4]

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Transported Stallion Semen and Breeding Mares with Cooled or Frozen-Thawed Semen

Sylvia J. Bedford-Guaus, DVM, PhD, Dip/ACT

Horse owners worldwide now request that their mares be bred with cooled or frozen-thawed transported semen, owing to the advantages of avoiding mare transport (often with a foal by her side), decreasing disease transmission between farms, and most importantly, the accessibility to a wider genetic pool. This has become commonplace practice as many breed registries now allow the use of transported semen for producing foals worthy of registration. However, problems arise as optimal steps for both semen handling and preparation, as well as for mare breeding management, are not practiced. Therefore, the objective of this manuscript is to provide veterinary practitioners with an overview about optimal management techniques related to both handling semen and mare management for attaining successful results. Common problems and dilemmas encountered will be also discussed and emerging research introduced. Whereas appropriate protocols for handling and preparing semen for cooled-transport will be presented with some detail, it is beyond the scope of this paper to discuss stallion semen cryopreservation, which typically requires extensive equipment investment and expertise and is usually done at referral and specialized centers. This text assumes that the equine veterinary practitioner already has some basic skills in regards to stallion semen collection and evaluation, as well as in performing examination of the mare via transrectal palpation and ultrasonography for breeding management purposes.
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KEYWORDS stallion, transported semen, artificial insemination, cooled semen, frozen semen

Cooled-Transported Semen: Definition and Basic Principles

For cooled-transport, stallion semen is diluted with an appropriate extender and packaged in a container that will facilitate slow-cooling to 4-6°C during shipment. It is important for practitioners to thoroughly understand this process so that breeding managers, often in charge, can be appropriately instructed. Additionally, suboptimal pregnancy rates in otherwise reproductively healthy mares may result from breeding with low-quality transported semen that was not adequately packaged for shipment; this provides undue stress to the veterinarian responsible for mare breeding, who is then confronted with an unhappy owner when the mare is open at the time of pregnancy check.

Extensive research has been published in the area of stallion sperm cooling, from the first report using the Equitainer™ (Hamilton-Thorn Research, Danvers, MA) as a cooling device suitable for semen transport.[1] For adequate results, it is imperative that semen be handled appropriately from collection through packaging to insemination. This includes care in preparation of the artificial vagina, which should be clean, dry, and not exposed to chemicals or disinfectants. Similarly, an appropriate nonspermicidal lubricant should be applied to facilitate penile intromission; in fact, exposure to a commonly used commercially available lubricant, KY Jelly® (Personal Product Company, Division of McNeil-PPC Inc., Skillman, NJ), has been shown to be detrimental to cooled stallion sperm motility,[2] most likely due to the presence of the bacteriostatic agent clorhexidine gluconate. Suitable alternatives for artificial vagina lubrication include HR Lubricating Jelly® (Carter Products, Division of Carter-Wallace Inc., NY), Priority Care Non-Spermicidal Sterile Lubricating Jelly® (First Priority Inc., Elgin, IL), or probably any sterile water-soluble lubricant devoid of bacteriostatic or other spermicidal compounds. It must be kept in mind that sperm are sensitive to all light, water, sudden temperature changes, and cleaning/disinfection chemicals. This should translate in always using disposable nonspermicidal supplies, or supplies that have been appropriately washed with enzymatic detergents and thoroughly rinsed with deionized water before air-drying. Similarly, to prevent cold-shock, all vessels and supplies to come into contact with freshly collected sperm should be kept in an incubator at body temperature (ie, 37°C).

Department of Clinical Sciences, Theriogenology Section, College of Veterinary Medicine, Cornell University, Ithaca, NY.
Address reprint requests to Sylvia J. Bedford-Guaus, DVM, PhD, Dip/ACT, Department of Clinical Sciences, College of Veterinary Medicine, Box 34, Cornell University, Ithaca, NY 14853. E-mail: sjb55@cornell.edu

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Table 1 Commercially Available Extenders Suitable for Cooled-Transported Stallion Semen

Product Name	Antibiotic Choices	Commercial Source
E-Z Mixin®	Amikacin sulfate Polymixin-B K-Penicillin-G + Amikacin sulfate Ticarcillin No antibiotic option*	Animal Reproduction Systems, Chino, CA; 800-300-5143; www.arssales.com
Universal Extender†	K-Penicillin + Amikacin sulfate Timentin No antibiotic option	Exodus Breeders Corporation, York, PA; 877-396-3874; www.exodusbreeders.com
Dr. Kenney's Extender	K-Penicillin + Amikacin sulfate Timentin No antibiotic option	
Kenney Extender	Not included	Hamilton Research Inc., South Hamilton, MA; 800-367-0266; www.equitainerstore.com
Skim-milk Extender	Gentamicin sulfate Ticarcillin disodium No antibiotic option	Lane Manufacturing Inc., Denver, CO; 800-777-2603; www.lane-mfg.com
Equipro®†	Gentamicin sulfate K-Penicillin + Amikacin sulfate Ticarcillin No antibiotic option	Minitube®, Verona, WI; 800-646-4882; www.minitube.com

*An antibiotic should always be added when storing stallion semen for cooled shipment. The "no antibiotic" option gives the purchaser the choice of acquiring the desired antibiotic from a different source.
†This extender contains sucrose in addition to glucose (ie, double sugar extender).

For cooled shipment, stallion semen is diluted in an appropriate prewarmed extender that preserves sperm viability over time by facilitating a source of energy, providing protection against temperature and pH changes, and diluting toxic byproducts released during cell death. The most commonly used diluent for cooled shipment of stallion semen is a nonfat dried skim milk–glucose (Kenney's) extender with the addition of suitable antibiotics.[3] This extender can be easily prepared in the laboratory (4.9 g glucose, 2.4 g dried skim milk, 100 mL deionized sterile water), but it is also available for purchase from several commercial sources (Table 1). Worth noting is that some of the commercialized semen extenders are prepared with the addition of sucrose (ie, double sugar extender[4]). There is no reported advantage of using the original Kenney's versus a double sugar extender, other than potential individual preferences for stallions with borderline semen quality. In this regard, a preseason cooled semen test should be performed in all stallions entering a transported semen program; ideally, their semen should be cooled with different extender (Kenney's versus double sugar) and antibiotic combinations (see below) to assess what works best for that particular stallion.

Research reports have also addressed the effect of antibiotics on sperm viability under cooled storage.[4-6] Present recommendations include the use of ticarcillin disodium (1 mg/mL), potassium penicillin (1000 IU/mL), or amikacin sulfate (1 mg/mL). Furthermore, it appears that the combination of the two latter antibiotics provides the best results in preventing bacterial growth while preserving sperm viability.[6] Conversely, antibiotics such as polymixin B sulfate or gentamicin sulfate have been shown to be detrimental for stallion sperm motility, in particular after cooled storage.[4,5]

Another crucial factor affecting sperm viability and fertility during cooled transport is the extender-to-semen dilution ratio. It is now well established that semen for shipment should be diluted at a final concentration of 25-50 $\times$ 10^6 sperm/mL, with an extender:semen dilution ratio of no less than 2:1 (vol:vol).[7,8] Therefore, appropriately extending semen for cooling requires the determination of the ejaculate's initial sperm concentration.

Several types of containers are commercially available for cooling equine semen during transport (Table 2). The most widely used shipping device is a reusable system (Equitainer I™ and Equitainer II™; Hamilton-Thorn Research, Danvers, MA) that provides a reliable cooling rate of -0.3°C/min initially, and then slower (-0.07°C/min) over the membrane lipid-phase transition (ie, cold shock) temperatures (19-8°C) deemed critical for stallion sperm.[9,10] Disposable containers, which basically consist of a cardboard box lined with insulating styrofoam and a cooling pack, are attractive to breeders since they avoid the shipping charges required to return the device to the farm of origin. Recent research has shown that most of these systems perform quite well in regards to cooling rate and final temperature (4-6°C) of stored semen as long as the container is kept at 22°C throughout the process.[9] The exception in this study was the ExpectaFoal™ system; extended semen packaged in this container while stored at room temperature reached temperatures below 0°C, and this negatively affected sperm motility after 24 hours of cooled storage. Furthermore, all containers performed suboptimally

Table 2 Commercially Available Containers for Cooled Shipment of Stallion Semen

	Source and Contact Information
Non-Disposable	
EST™-SH and EST XL™ SH*,†	Plastilite Corporation, Omaha, NE; 800-228-9506
Equitainer I‡ and II™§	Hamilton Research Inc., South Hamilton, MA; 800-367-0266
Disposable	
Bio-Flite™	Bio-Flite, Anaheim, CA; 714-921-2398
Clipper™*	Hamilton Research Inc., South Hamilton, MA; 800-367-0266
EST S™, EST XL S*,†	Plastilite Corporation, Omaha, NE; 800-228-9506
Equine Express™	Exodus Breeders Corporation, York, PA; 877-396-3874
ExpectaFoal™	Expecta, Parker, CO; 303-341-2268

*Not evaluated in study by Brinsko et al., 1999.
†EST™, Equine Semen Transporter; SH and S, holds temperature for up to 60 hours; XL, holds temperature for up to 120 hours.
‡Holds cooled storage temperature for up to 70 hours.
§Holds cooled storage temperature for up to 48 hours.

when stored at freezer (−20°C for 6 hours followed by storage at 22°C for 18 hours) or incubator (37°C for 24 hours) temperatures, although cooling rates and final storage temperatures were most affected in disposable containers, with once again the Expecta Foal™ container being the least reliable. These are important considerations when choosing a shipping container since often we have little or no control over the environmental temperatures at which these devices will be exposed during shipment. In all instances, it is imperative that semen packaging instructions provided by each system are followed accurately for optimal results.

Step-by-Step Evaluating and Packaging Stallion Semen for Cooled Transport

Because appropriate evaluation, handling, and packaging of cooled semen is a critical step for achieving optimal pregnancy rates, this will be covered in some detail. Once an ejaculate has been collected, semen volume, sperm concentration, and percentage of progressively motile sperm should be evaluated for every sample to be shipped.[11] The author also recommends that at least once at the beginning of the breeding season the sperm from a stallion entering a cooled-semen program be evaluated for percent morphology; it is not necessary to repeat this throughout the breeding season unless warranted by a sudden deterioration in other sperm quality measures or a decrease in pregnancy rates. For semen evaluation purposes, every breeding operation should have a temperature-controlled laboratory equipped with an incubator, disposable semen containers (graduated cups or cylinders), a microscope, and means of evaluating sperm concentration. The following are suggested steps for evaluation of the semen sample.

Appearance of the Sample

This mostly refers to color. Semen normally is whitish to grayish in color and creamy to translucent, depending on the concentration of sperm. For example, a yellow sample may indicate contamination with urine, and a pink or brownish sample may indicate contamination with blood, both of which will be detrimental to sperm. A transparent sample may indicate that only seminal fluid, and no sperm, was ejaculated. Any clumps, shreds, or other debris should be noted.

Volume

Immediately after collection, semen is typically filtered to remove the gel fraction and any debris present into a disposable prewarmed graduated plastic cup or cylinder. Decanting semen into a graduated vessel allows for determination of semen volume.

Concentration

The concentration of sperm in the sample can be measured by means of a hemacytometer, or commercially available spectrophotometers specially calibrated to count stallion spermatozoa (Densimeter®; ARS, Chino, CA; SpermaCue™, Minitube®, Verona, WI). Concentration is given in millions of sperm per milliliter of semen.

Total Number of Sperm

The total number of sperm is calculated by multiplying the number of sperm per milliliter (or concentration) times the volume of the sample. The total number of sperm is traditionally expressed in billions. Given good sperm quality measures, the total number of sperm at daily sperm output in the ejaculate is the single most important characteristic since it will determine the number of mares that can be bred by artificial insemination with fresh or cooled semen.[11]

Percentage of Motile Sperm

The percentage of motile sperm is evaluated by placing a drop of semen on a warm microscope slide and a warm cover slip, and observing it under the microscope at 200× magnification. Two types of sperm cell motility are usually estimated by looking at least at 10 different fields on the slide; total motility is the percent of sperm that are just moving, in any form or direction, whereas progressive motility includes only the percent of sperm moving in a seemingly straight line.

Total Number of Progressively Motile Sperm

This is calculated by multiplying the total number of sperm by the percent of progressive motility. A breeding dose with fresh or cooled semen should contain a *minimum* of 500 million progressively motile spermatozoa.[12,13] To account for sperm death during transport, a minimum of 1 billion progressively motile sperm should be shipped for each mare to be bred with cooled semen. Beware that this is just a minimum! Misinformation regarding problems with large insemination volumes resulting in decreased fertility in mares may influence some breeders to ship the minimum number of sperm and throw the rest away. Although this is further explained in a later section, in my experience, optimal preg-

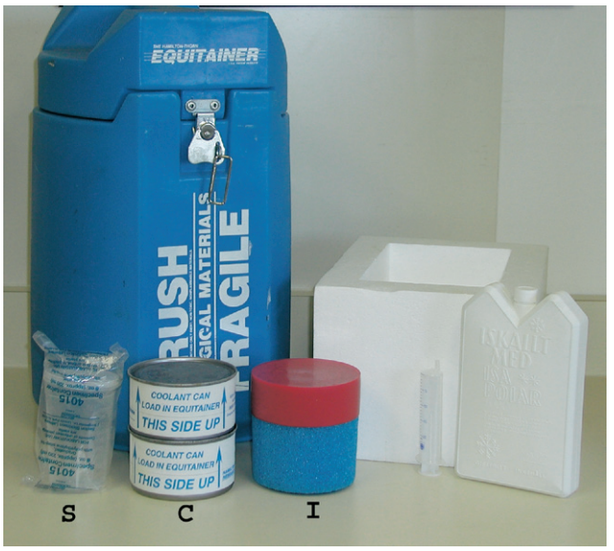


Figure 1 Devices used to ship cooled stallion semen. Equitainer™ system (left) and sample styrofoam box used in disposable containers (right). S, sample cup; C, coolant cans; I, isothermalizer. (Color version of figure is available online.)

nancy rates are achieved by breeding mares with unlimited sperm numbers and large extended semen volumes,[14] provided good initial sperm quality and as long as appropriate guidelines for handling semen and for breeding management are followed accurately.

Percent Morphologically Normal Sperm

The evaluation of sperm morphology is performed either on a wet mount under phase contrast microscopy or on an air-dried sample stained with eosin/nigrosin under light microscopy (1000×). Please note that the latter is also a vital stain, thus only dead sperm (ie, sperm with a compromised plasma membrane) will pick up eosin stain and thus appear pink, whereas the outline of live sperm will be visualized over a dark background. At least 100 cells are typically evaluated, and the presence of the different morphological anomalies is noted. Serious concerns arise when a stallion yields ejaculates with less than 50% morphologically normal sperm.

When performing these measures of semen evaluation, time is of the essence as sperm quality deterioration will unavoidably occur in raw semen samples over time. Accurate but expedited semen evaluation requires practice; therefore, if the semen evaluation cannot be performed in 5 minutes or less, I recommend that an aliquot of the semen sample be separated for evaluation and the rest of the ejaculate can then be extended at a 1:1 (vol:vol) ratio until the decision is made as to how much extender to add to reach an appropriate final sperm concentration for shipment.

One controversial issue in reference to semen handling during the initial evaluation and while calculations for addition of extender are performed is whether raw semen should be placed in an incubator (ie, 37°C) or left on the counter top at room temperature (ie, 21-24°C). Although there is no controlled study looking at the effects of short-time storage of semen at 37°C, extended semen samples kept at this temperature for only 6 hours displayed a total sperm motility of 5% (compared with 82% for samples slowly cooled and stored at 4°C[15]). For this reason and because semen temperature probably starts to decrease immediately after collection, the author strongly recommends that semen be kept at room temperature while the initial evaluation is being performed. Along the same lines, once semen has been collected, the prewarmed extender should be immediately removed from the incubator, so that its temperature slowly decreases to match that of the semen sample. Naturally, this assumes that work is being performed in a laboratory with controlled ambient temperature (21-24°C) and no cold drafts.

As previously stated, cooled shipment is performed by packaging appropriately extended semen into a specially designed container that allows slow cooling while keeping the sample isolated from the external environment. Because the Equitainer™ is the most widely used nondisposable semen shipment container in the US, instructions of how to package semen for cooled shipment in this system will be described in detail. The Equitainer™ system includes (Fig. 1): (a) The Equitainer or "blue container" itself; (b) the isothermalizer, which is the device that is in contact with the coolant cans and that holds the semen cup; (c) the semen cup (disposable), which fits inside the isothermalizer; (d) coolant cans, which are fluid-containing cans that are frozen before use thus providing the basis for cooling the semen sample; and (e) ballast bags, to provide an appropriate volume within the semen containing cup. One important factor that is often overlooked is the volume of extended semen to be packaged within the sample cup; as per Equitainer™ system instructions, this volume should range between 120 and 170 mL (cc) to ensure a reliable cooling rate and optimal final temperature. If a minimum of 120 mL of extended semen is not available for shipment, then a ballast bag is placed in the sample cup surrounding the bagged semen sample to reach an appropriate final volume for cooling purposes. Ballast

Figure 2 Extended semen has been double bagged into baby bottle liners fastened using braiding rubber bands. A 60-mL ballast bag provided with the Equitainer™ system is shown. The ballast bag should be used to bring up the total liquid volume packaged within the Equitainer to a minimum of 120 mL. This will ensure that extended semen does not cool too fast during shipment and that it reaches a final temperature of 4-6°C. (Color version of figure is available online.)

Figure 3 Removing air from a Whirl-Pak bag containing extended semen ready for cooled shipment. Sliding the bag gently against the counter top allows for complete air removal. The wire edge of the bag should be cut-off before fastening the bag with braiding rubber bands or a heat sealer. (Color version of figure is available online.)

bags (60 mL each, filled with purple fluid) can be purchased with the Equitainer™ system (Fig. 2). Otherwise, an appropriate volume of water or extender can be placed in an adjacent bag to function as a "ballast bag," as long as it is appropriately labeled as "do not use for insemination." Importantly, the coolant cans should always be completely frozen to −20°C before packaging semen in the Equitainer™, and should have been kept in a deep freezer for at least 24 hours before use; this applies also for any cooling pack used in other shipping devices. Conversely, the semen holding cup and isothermalizer should be kept at room temperature before use.

Once semen has been properly extended, an appropriate volume for shipment is placed into commercially available Whirl Pak® bags[1] (Fig. 3) or baby bottle liners (Fig. 2); semen is typically double bagged to minimize leakage during shipment. If using baby bottle liners, one should be aware that some plastic brands might be spermiotoxic and this should be suspected if sperm quality decreases significantly during cooled storage. Before closing these plastic bags, air should be removed to minimize oxidative damage of sperm (Fig. 3).[16] Bags might be then fastened with braiding rubber bands (Fig. 2) or with a heat sealer, being careful not to heat the extended semen in the process. In the case of Whirl Pak® bags, I recommend cutting off the wire end to avoid puncture of the bag and leakage of the sample during shipment. Appropriately fastened bags are then placed in the sample cup and packaged in the cooling container for transport, always taking into account the appropriate final volume to be shipped (120 to 170 mL in the case of the Equitainer). The sample cup is then placed in the isothermalizer, and the whole unit (isothermalizer with sample cup) is stacked on top of and in direct contact with appropriately frozen coolant cans.

Although basic principles of evaluating and extending semen for cooling are the same for any shipping container used, there is some variability as to the actual semen-containing vessel. In some of the disposable cooling containers, extended semen is drawn into syringes and placed in the container in indirect contact with a cooling pack. If semen is to be shipped in syringes, these should have plastic rather than rubber plungers which might release toxic substances that are spermicidal (Fig. 4).[17,18] Other systems and specially designed isothermalizers for the Equitainer™ system require a 50-mL conical centrifuge tube for packaging semen for shipment. In all cases, it is imperative to follow appropriate guidelines for extending semen and to avoid leaving any air pockets that will invariably accelerate oxidative sperm damage and membrane lipid peroxidation.[16]

Last but not least, before closing the shipping container, it is highly advisable to always include a form with the stallion's name and detailed information regarding semen evaluation measures, extender used, as well as final sperm concentration and total number of sperm shipped. In fact, practitioners at the receiving end should insist that a semen information form be included at the time of shipment, not only to compare initial findings with the final product, but also to ensure that breeding is being performed to the appropriate stallion. Transported semen is typically shipped by next day courier service or same day by air in a counter-to-counter service. The latter is more expensive and requires a trip to the airport, but useful when a mare is very close to ovulation or if the stallion semen quality will not survive 24 hours of cooled storage. At the mare's end, the shipping container should not be opened until the mare is in the premises, scrubbed, and ready to be inseminated.

Cooling Semen from Stallions with Low Sperm Concentrations in the Ejaculate

One problem that practitioners in this field and breeding managers sometimes encounter is that of shipping semen from stallions producing ejaculates with initial low sperm concentrations not suitable for dilution at the appropriate concentration and extender dilution ratio recommended for cooling. Worth noting is that, in these cases, the sperm quality is often borderline, and thus sperm might not survive the cooling process. The problem of low initial concentration

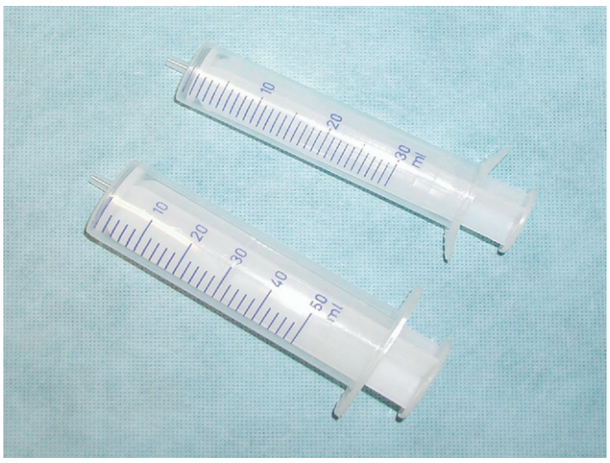


Figure 4 Samples of two sizes of all-plastic syringes used for cooled semen shipment in some disposable systems and for insemination. (Color version of figure is available online.)

might be overcome by centrifuging the ejaculate and partially removing seminal plasma, followed by extending the sperm pellet at an appropriate concentration for cooled shipment. A disadvantage is that sperm might be lost and/or damaged during the centrifugation process. Additionally, it requires the purchase of an expensive piece of equipment and some understanding about the principles of appropriately centrifuging semen. However, a recent study showed that semen from "poor cooling" stallions performed better after cooled storage when 90% of the seminal plasma was removed and the remaining semen pellet was extended in amikacin containing skim-milk glucose extender at 25-50 $\times$ 10^6 sperm/mL for cooled storage.[19] In this study, semen was centrifuged at 400 g for 12 minutes.

An alternative to obtain more concentrated ejaculates in some stallions is semen fractionation at the time of collection. This requires the use of an open-ended artificial vagina and the collection of the first three to four (sperm-rich) ejaculatory jets. In one report, sperm motility of sperm-rich fractions was higher than the motility of sperm from a complete ejaculate after cool storage for 24 and 48 hours.[7] Therefore, it might be worthwhile to perform ejaculate fractionation in stallions with full ejaculates containing a low sperm concentration, especially if their sperm motility decreases significantly during cooling.

The Basics of Frozen-Thawed Stallion Semen

This is semen that has been appropriately processed in extender that contains cryoprotectants and packaged in straws for storage in liquid nitrogen (−196°C). Therefore, if appropriately stored, frozen semen will theoretically last forever! There are numerous freezing protocols presently being applied to stallion semen,[20] some of which are proprietary information. Additionally, frozen stallion semen might be packaged in 0.5- or 5-mL straws. One known disadvantage of the larger straws is that, during freezing, the entire surface area of the straw is not uniformly exposed to the same temperature gradient, thus potentially compromising cooling rate and sperm viability.[20,21] An advantage, however, is that less straws will be required to achieve appropriate insemination dose, which makes thawing and handling at the time of insemination less cumbersome. In any case, it appears that most authors presently agree that 0.5-mL straws provide a more uniform freezing rate, and thus better fertility results than 5-mL straws.[20,21]

Although the process of freezing semen will not be covered in this manuscript, from the user's point of view it is very important to understand that every freezing protocol and packaging system calls for a particular thawing method.[22] Therefore, frozen semen should always be provided with an appropriate thawing procedure and this followed accurately for best results. However, even when specific instructions are followed and under optimal breeding management conditions (see below), it is well recognized that fertility with frozen-thawed stallion semen is questionable and, furthermore, stallion-dependent.[20,22] Thus, it is very important that owners are appropriately informed before making the decision of breeding their mare with frozen-thawed stallion semen.

General Considerations for Breeding Management with Transported Semen

Optimal pregnancy rates with preserved semen can only be achieved when both stallion and mares in the program are reproductively sound. Appropriate handling of semen for cooling purposes was covered above. Similarly, frozen semen must be chosen from reliable sources and, if possible, we must strive to convince owners to purchase frozen semen from stallions that have produced foals using frozen semen. Additionally, optimal breeding management by the veterinarian at the mare's end is as important as appropriate handling of semen at the point of origin.

An additional consideration for cooled-semen programs is that semen from some stallions is only collected on scheduled days, typically on Mondays, Wednesdays, and Fridays. This is complicated further when stallion managers request a 24-hour advanced notice for semen shipment. These factors can make the decision of when to order semen tricky, and thus good communication at both ends is of utmost importance. This is not as critical for frozen-thawed semen since an appropriate number of straws might be shipped before the mare comes into estrus as long as a liquid nitrogen tank is available for storage at the mare's end.[22] Dry shippers used to transport frozen semen can also hold a certain number of straws for a limited amount of time (ie, 7-14 days); thus, this it is a good alternative for short-term storage if the semen is ordered right around the time when the mare comes in heat.

The literature is full of research reports using cooled stallion semen for breeding with pregnancy rates achieved ranging from 31% to 96%.[1,14,23,24] Although certain recommendations might vary among authors, it is generally in agreement that, with cooled-transported semen, mares should be bred within 48 hours before ovulation for optimal results. Often, two doses of semen are sent in one shipment. In this regard, one controversial issue is that of whether it is best to breed mares with the two doses at once, or store one dose at 5°C and breed the mare again 24 hours later. Two studies have addressed this question.[23,25] In one study, three groups of mares were bred: (1) twice with 1 $\times$ 10^9 sperm from the same collection at 24 and 48 hours after cooled storage; (2) once with 1 $\times$ 10^9 sperm cooled for 24 hours; or (3) once with 2 $\times$ 10^9 sperm cooled for 24 hours.[23] All mares were given human chorionic gonadotropin (hCG) at the time of the first insemination and ovulated within 48 hours. When pregnancy rates were pooled for all three stallions used in the study, these were 64%, 31%, and 41% for the three groups of mare treatments, respectively. Glancing at these results, it would be easy to conclude that breeding twice, 2 days in a row, at approximately 48 and 12-24 hours before ovulation with two different doses from the same semen shipment provides the best pregnancy results. However, this study has some flaws: most notably, only for one of the three stallions in the study were there significant differences among the three mare treatment groups. Furthermore, for this particular stallion, pregnancy rates were extremely low ($\leq$21%) for mares assigned to the single breeding groups; higher pregnancy rates should be expected in a research setting using fertile mares and stallions. Similarly, for all stallions combined,

pregnancy rates across all treatments averaged only 45%. In a different study, two groups of mares were bred either: (1) once, with semen cooled for 24 hours containing 500×10^6 progressively motile sperm; or (2) twice, with 250×10^6 progressively motile sperm each time, cooled for 24 and 48 hours, respectively.[25] Pregnancy rates were the same for mares in the two treatment groups (12/18, 67%). Based on these results, either regime might be acceptable for stallions whose semen performs well under long-term cooled storage; however, breeding once when the shipment is received is generally preferable as for most stallions sperm quality deteriorates rapidly beyond 24 hours of cooled storage. An additional important factor to take into account in this regard is that breeding every 24 hours is contraindicated in mares with poor uterine clearance predisposed to postbreeding endometritis; in fact, every effort should be made to minimize the number of inseminations per cycle in such problem mares.[26]

Regarding use of frozen-thawed semen, reported pregnancy rates are variable and, as previously mentioned, highly stallion-dependent.[20] Recommendations for breeding with frozen-thawed semen vary from insemination within 6 to 12 hours before and/or after ovulation.[24,27,28] Whether one or two (typically one before and one after ovulation) inseminations are performed often depends on the number of straws and corresponding breeding dose allocated per mare cycle in the stallion contract. Similarly, number of straws and corresponding number of sperm to be used for insemination is often set by the facility freezing the semen; therefore, the veterinarian inseminating the mare has little to no control over this factor.

Insemination Dose and Volume

Numerous studies have addressed what is an appropriate dose when using fresh or cooled sperm for artificial insemination.[12,13,29] Although pregnancies have been achieved using as low as 100×10^6 progressively motile sperm, it is widely accepted that at least 500×10^6 progressively motile sperm should be inseminated to optimize pregnancy rates.[12,13] Therefore, at least 1×10^9 progressively motile sperm should be shipped to ensure that a minimum breeding dose will be available after 24 hours of cooled storage.

Unfortunately, there is a widespread tendency to limit the amount of cooled sperm shipped, even when the whole ejaculate is not being used. At the stallion's end, this means shipping one or two billion progressively motile sperm and discarding the rest down the sink. This practice stems partially from the misconception that large insemination volumes might adversely affect pregnancy rates in mares. In one study, large insemination volumes (100 mL) resulted in significantly lower pregnancy rates than small volumes (10 mL).[30,31] However, in this particular study, the total number of inseminated spermatozoa was fixed at 250×10^6 progressively motile sperm. There is one remarkable problem with the experimental design in this study: the larger insemination volume used (100 mL) contained a final sperm concentration of only 2.5×10^6 progressively motile sperm/mL, which is known to be too dilute for adequate fertility. Following this study, other authors then compared insemination volumes of 10 and 50 mL while maintaining the concentration of sperm constant at 25×10^6 sperm/mL; in this case, embryo recovery rates were not different between the two insemination volumes.[8] From the combined results of these two reports, it was concluded that insemination volumes over 50 mL adversely affect fertility in mares.[32] This unfounded dogma has unfortunately lingered within the equine breeding industry.

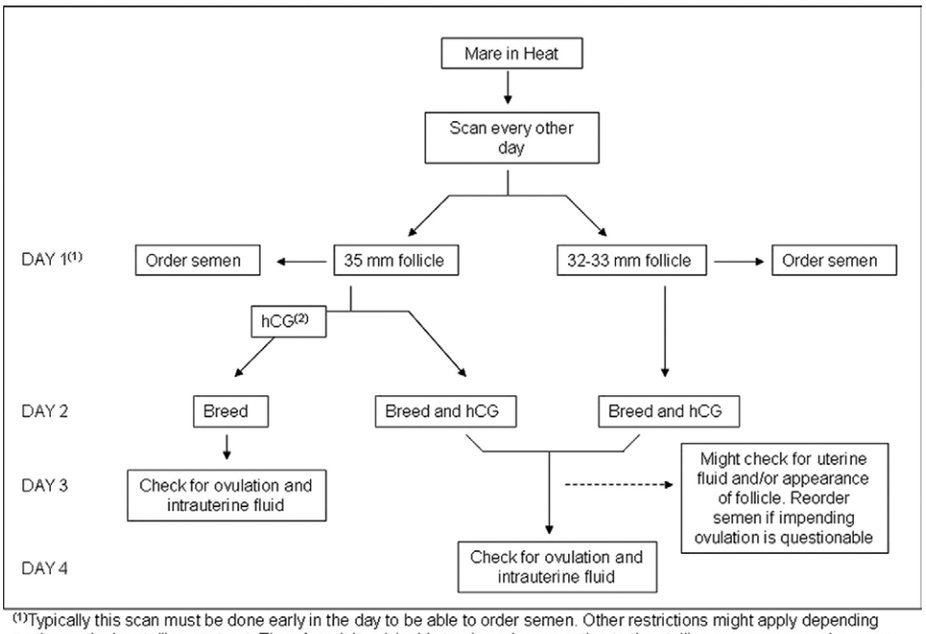
To appropriately address this issue, we performed a study comparing insemination volumes of 30 and 120 mL in a crossover randomized trial using 10 pony mares.[14] Semen was extended at 50×10^6 sperm/mL and inseminated after 24 hours of cooled storage under optimal breeding management conditions. Overall, 7/9 and 10/10 mares became pregnant with the 30- and 120-mL treatment, respectively. We concluded that large insemination volumes do not have an adverse effect on fertility. Furthermore, one argument against the use of large insemination volumes is that it might predispose mares to develop postbreeding endometritis.[30] In our study, only one mare pooled large amounts of fluid in the uterus and this was after she had been inseminated with the 30-mL treatment, further supporting that initial insemination volume is not the trigger for pooling intrauterine fluid postbreeding. Moreover, Kotilanen and coworkers[33] showed that it is actually the concentration of sperm in the sample rather than the actual volume inseminated that determines the degree of inflammatory reaction in the mare's uterus. In fact, extending semen and inseminating a larger volume (at a minimal concentration of 25×10^6 sperm/mL) should have a beneficial effect against postbreeding endometritis.

With frozen-thawed stallion semen, volume is not an issue, as usually very small volumes (1-5 mL) with high sperm concentrations are used for insemination. Precisely for this reason, susceptible mares bred with frozen-thawed semen should be closely monitored for postbreeding uterine fluid accumulation and managed accordingly.[26,33] For frozen semen, the optimal breeding dose in regards to number of sperm to be inseminated is not as standardized as it is for cooled-transported semen. Reported insemination doses with frozen-thawed semen range from 250×10^6 to 800×10^6 progressively motile sperm.[28,34] Therefore, the number of straws required to breed a mare will depend on the sperm concentration at the time of freezing and the postthaw motility; this would be determined by the facility freezing the semen and should be included in the stallion contract and in the instructions provided with the semen shipment.

Strategies for Breeding Mares with Transported Semen

Breeding mares with either cooled or frozen-thawed semen requires close monitoring of the estrous cycle. If a stallion is available on the premises, cycling mares might be teased every other day to detect heat. If a stallion is not available, heat detection must be performed by the veterinarian based on findings from transrectal examination of the reproductive tract. In either case, once a mare is in heat, follicular growth must be monitored by ultrasound per rectum to appropriately plan for breeding timing.

To inseminate at the optimal time, it is advisable to use hormones that will hasten ovulation. This will also help minimize the number of days for which close monitoring is re-



(1)Typically this scan must be done early in the day to be able to order semen. Other restrictions might apply depending on the particular stallion contract. Therefore, it is advisable to give advance notice to the stallion manager once the mare comes in heat.
(1)Deslorelin acetate can be given instead

Figure 5 Flowchart exemplifying typical protocols for successfully breeding mares with cooled-transported semen.

quired as well as the number of inseminations per cycle.[35,36] There are presently two hormones commonly being used to induce ovulation in mares: hCG and the gonadotropin-releasing hormone (GnRH) analog deslorelin acetate. The latter can be given to mares in two formulations: a controlled release subcutaneous implant (Ovuplant®; Fort Dodge Animal Health, Overland Park, KS; no longer available in the USA) or a short-term release liquid form (BioRelease Deslorelin injection; BET Pharm, Lexington, KY) which is administered as a single injection (1 mL, IM). Human chorionic gonadotropin or deslorelin should be administered when a preovulatory follicle reaches a minimum size of 35 mm in diameter; a high percentage of mares will ovulate within 48 hours of hormone administration.[35,37,38] Because hCG is a large molecule, it induces an immune response[39] and thus there is a concern for decreased efficiency after repeated use; however, results in this regard are controversial.[36,40] The author will use deslorelin in breeding mares for which hCG has not yielded predictable results in a previous cycle.

As for cooled-transported semen, there are many potential breeding regimes that will provide adequate fertility rates. In general, as stated above, the goal is to breed within 48 hours before ovulation. For this purpose, one potential regime is to order semen once a follicle reaches 35 mm in diameter. Either hCG or deslorelin can then be administered on the day that the semen is ordered or on the day of insemination. In either case, a mare that responds to the hormonal treatment should ovulate within 48 hours of insemination. One potential disadvantage of administering the hormone on the day that the semen is ordered is that occasionally semen shipments are lost in transit; in such an instance, there might not be time to reorder semen before the mare ovulates and that estrous cycle will be missed. Since a dominant follicle will grow approximately 2 to 4 mm/day, another potential regime is to order semen once a follicle reaches ≈33 mm in diameter. Once the shipment arrives, 24 hours later, the follicle should have reached 35 mm and thus insemination and ovulation induction will be performed on the same day. A flowchart summarizing common breeding regimes with cooled transported semen is presented in Fig. 5. These regimes strive for breeding once with a single semen shipment; however, one might also plan for two shipments, especially in mares that have a history of ovulating relatively small follicles (ie, <35 mm in diameter). For instance, a mare might be bred once before the dominant follicle reaches preovulatory size, and if she has not ovulated 48 hours later, the mare can be bred again with a second semen shipment once the follicle will respond to hCG or deslorelin. Thus, mares ovulating before reaching the 35-mm mark will not be missed. However, this protocol would be less advisable in older mares with poor uterine clearance predisposed to postbreeding endometritis, as a second insemination will further enhance the inflammatory response of the uterus.[26]

As stated above, success with frozen-thawed semen requires that insemination be performed much closer to ovulation, typically within 12 to 6 hours before and/or after ovulation.[24,27,28] If only one breeding dose is available per cycle, the only possibility is to scan the mare's reproductive tract once daily until the dominant follicle reaches 35 mm in diameter; then hCG or deslorelin is administered and scanning is performed four times daily (approximately every 6 hours) until ovulation is detected, at which time the mare is bred. This will ensure that an insemination is performed within 6 hours after ovulation. It is very effective but time consuming, difficult in private practice, and expensive for the mare's owner. Probably these are some of reasons why many practitioners in the field breed within 12 hours of ovulation (before and/or after), which would require checking the mare only twice daily once hCG or deslorelin has been administered[41]; however,

breeding close to or beyond 12 hours postovulation might compromise fertility results. When two insemination doses are available per mare cycle, one dose can be administered before the mare ovulates (ie, when ovulation is deemed imminent or about 24 hours after ovulation induction), and the second dose after ovulation is detected; with this protocol it is likely that one of the inseminations will have been performed close enough to ovulation to achieve adequate pregnancy results. Overall, these protocols might yield pregnancy rates ranging from 30% to 50%.

To overcome the frequency of mare examination by ultrasonography required to follow the above regimes, a simplified timed strategy has been reported.[28] Following a protocol whereby mares were bred at 24 and 40 hours after administration of hCG with 400×10^6 frozen-thawed spermatozoa (percent motility was not reported), pregnancy rates were similar (55%) than those obtained when mares were bred with 800×10^6 spermatozoa only once within 6 hours after ovulation (60%); the latter regime required checking mares four times daily. In another report, pregnancy rates were 76% versus 71% for two timed inseminations versus breeding once within 6 hours after ovulation, respectively.[42] The timed strategy is very attractive but it does require two breeding doses per cycle, which are not always provided. Furthermore, to trust a timed insemination protocol, hCG must be administered just around the time when a 35-mm follicle is first detected; if hCG is given when a follicle is already much larger than 35 mm in diameter, then one cannot be certain that this follicle will not ovulate spontaneously before the 36- to 44-hour interval typical for a response to the exogenous hormone.

Insemination Technique

When using artificial insemination, mares should always be bred following the minimal contamination technique described by Kenney and coworkers.[3] Briefly, the tail should be wrapped and tied to the restraint stocks or to the mare's neck. The perineal region should be thoroughly scrubbed with povidone iodine; typically three alternate scrubs and rinses followed by thorough rinsing with clean water to remove wash residues is sufficient. The perineal area should then be dried with clean soft paper towels.

Only after the mare has been examined and the perineum prepared for breeding should the semen sample be prepared for insemination. For cooled-transported semen, the shipping container is opened; I typically glance at the enclosed information sheet and quickly check sperm motility, as a sample containing only nonmotile sperm should not be inseminated. Worth noting is that cool sperm might swim slowly and appear "sluggish"; this is normal as long as sperm activation becomes apparent as the temperature of the sample increases. A small aliquot can be removed for later evaluation. Importantly, the semen sample should not be warmed up before insemination. After the mare has been inseminated, I always perform a complete semen evaluation as explained earlier in this manuscript. Sperm concentration of extended semen must be performed using a hemacytometer, as extender components will interfere with spectrophotometer-based readings.

For frozen semen, thawing instructions should have been read in advance and a water bath should have already been set at the appropriate thawing temperature. Thawing temperature depends on the freezing method, and might range anywhere from 37°C to 70°C.[20,22] When using 37°C, a minimum amount of time is usually required for complete thawing, but a slight delay in removing the straw from the water bath is usually not detrimental. However, when thawing straws at higher temperatures it is extremely critical that the number of seconds designated in the thawing protocol be followed exactly, as longer exposure to high temperatures will damage sperm[22] and negatively affect pregnancy rates. When straws are removed from the water bath, they should be dried with a clean paper towel to avoid water drops leaking into the semen sample once the straws are cut open. The contents of the straw can be emptied into a small plastic centrifuge tube by cutting both straw ends; the thawed semen is then drawn into a syringe for insemination. As for cooled semen, I always examine a small drop of semen under the microscope for sperm motility; a minimum of 30% progressive motility is considered acceptable for frozen-thawed stallion sperm.[20] The small amount of thawed sperm available for insemination usually limits the possibility of setting a sample aside for concentration or morphology evaluation.

To inseminate the mare, a sterile plastic sleeve should be placed over the arm and lubricated with a small amount of nonspermicidal sterile jelly. The tip of a sterile insemination pipette is then cupped within the sleeved hand and carefully inserted through the vulva, vestibulo-vaginal sphincter, and into the vagina, with the index finger identifying the cervical os to guide the pipette into the uterine body. As discussed earlier, syringes used for insemination should have a solid plastic plunger.[17,18] Performing a clean step-by-step procedure should minimize the amount of contamination introduced at the time of breeding and thus reduce the chances of infection, in particular in susceptible mares.[3,26]

One important tip to keep in mind when breeding mares with frozen-thawed semen is that, due to the small insemination volume, one must make sure that enough air is pushed through the insemination pipette to deliver the entire breeding dose into the uterine lumen. In this regard, it has been shown that depositing frozen-thawed semen deep into the uterine horn ipsilateral to the preovulatory follicle provides no advantage in regards to pregnancy rates over placing the inseminate within the uterine body.[28]

Conclusions

Breeding mares with transported semen requires both a good understanding of appropriate semen handling and processing techniques, as well as good practice of breeding management. Understanding how semen must be processed and packaged for cooled shipment can allow the veterinary practitioner to: (1) collect and ship stallion semen to other facilities; (2) instruct owners and breeders about optimal semen handling and packaging techniques; and (3) judge whether appropriate methods have been followed when examining shipped semen at the receiving end so that the required changes can be implemented for future shipments. When using cooled or frozen-thawed stallion semen for breeding, mares must be followed closely for follicular growth and

hormonal treatments should be applied to time breeding with ovulation. For optimal breeding management, cooled semen should be inseminated within 48 hours before ovulation, whereas frozen-thawed semen should be inseminated within 12 hours before and/or 6 hours after ovulation. Pregnancy results will depend on the inherent fertility of the mare and stallion, the use of adequate handling and processing semen techniques, and the application of optimal breeding management strategies.

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Evaluation of Stallion Sperm Morphology

Leonardo F. C. Brito, DVM, MSc, MVetSc, PhD, DACT

Evaluation of sperm morphology is an important part of stallion breeding soundness evaluation. The objectives of this review are to discuss the pathogenesis of sperm defects and describe some of the sperm abnormalities present in the ejaculate of stallions.
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KEYWORDS stallion, sperm morphology, sperm defect, breeding soundness, fertility

Unlike other domestic livestock species, stallions are seldom selected for breeding solely on reproductive performance. Breeding stallions are selected based on their pedigree, athletic performance, or other phenotypic characteristics. In horses, if an end of season pregnancy rate of 80% to 75% and a foaling rate of 70% to 60% are considered satisfactory, this can be achieved within 4 cycles per mare by a stallion with a per cycle pregnancy rate as low as 35%.[1] However, changes in the equine industry indicate that the importance of more critical fertility evaluation is increasing. A recent report indicates that the mare book of Thoroughbred stallions in North America has increased considerably in the last 15 years, and that in 2005, 11% of stallions had a book greater than the traditional book of 40 mares. More importantly, over 50% of Thoroughbred mares were bred by stallions with books greater than 40 mares, and 35% of the mares were bred by stallions with books greater than 80 mares. Interestingly, foaling rates increased as the book increased, indicating that selected fertile stallions are able to couple with the larger books.[2] The increasing use of artificial insemination with cooled transported semen allowing stallions to breed hundreds of mares during one season, and especially the increase in the use of frozen semen commonly sold as a breeding dose without any fertility guarantee, have also placed more emphasis on the importance of semen evaluation.

Evaluation of sperm morphology is part of the stallion breeding soundness evaluation. The purpose of a stallion breeding soundness evaluation is to identify those stallions that can be expected to be incapable of achieving specific minimum levels of fertility, or at least alert owners of potential problems. In addition, the examination assists in identifying potential causes of reduced fertility so that measures can be taken to maximize stallion fertility.[3] It is important to realize that, due to the extreme complexity of the biological process involved in fertilization, the possibility of ranking stallions according to their fertility based on semen evaluation is a goal that might never be achieved. It is crucial to understand that the main objective of semen evaluation is to identify infertile and subfertile stallions (or ejaculates), ie, those of which fertility is expected to be <40% of the average for stallions of the same type (breed, age).[4] "An examination of sperm morphology alone can never justify the statement that the potential fertility of an ejaculate is high, but is reasonable to state that potential fertility is low when a high proportion of spermatozoa have abnormalities."[5]

The most widespread (and dangerous) misconception regarding evaluation of stallion sperm morphology is the assumption that morphology is not important or that it is less important than sperm motility just because few reports failed to demonstrate a relationship with fertility or the relationship with motility was of greater magnitude. As recently reviewed, the majority of studies involving evaluation of fertility in horses present limitations of the experimental design (eg, reduced number of stallions or mares, use of different fertility endpoints, etc.) that makes the interpretation of the data difficult and the conclusions questionable.[6] Sperm morphology greatly impacts fertility in all species studied, and it would not be expected to be any different in horses. The objectives of this review are to discuss the pathogenesis of sperm defects and describe some of the sperm abnormalities present in the ejaculate of stallions.

Pathogenesis of Sperm Defects

The ability of veterinarians to associate the knowledge of physiology and pathology with the stallion history and clinical findings to establish the prospective fertility of a stallion and to prognosticate the potential changes in fertility with changes in management or clinical treatments is what differentiates sperm morphology evaluation from a simple technical procedure. Therefore, it is imperative that veterinarians have a comprehensive understanding of spermatogenesis and pathogenesis of sperm defects.

Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA.
Address reprint requests to Leonardo F. C. Brito, Department of Clinical Studies New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348. E-mail: lfcbrito@lycos.com

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Spermatogenesis is the chronological, organized process involving the multiplication and differentiation of the germ cells in the seminiferous epithelium of the testes that results in the formation of a highly specialized cell, the spermatozoon, which constitutes the male gamete.[7,8] Spermatogenesis can be divided into spermatocytogenesis, meiosis, and spermiogenesis. Spermatocytogenesis is the cyclic proliferation of the germ stem cell population of spermatogonias by mitosis to produce primary spermatocytes and at the same time to renew their own number and continue the lineage of stem cells. Spermatocytogenesis lasts 19.4 days in stallions. Meiosis consists in the formation of secondary spermatocytes and haploid spermatids from the diploid primary spermatocytes and lasts 19.4 days in stallions. Spermiogenesis consists in the metamorphosis of haploid round spermatids into mature sperm that have a small flat head with a condensed nucleus and a specialized vesicle containing enzymes, and a tail that is necessary for motility. Spermiogenesis does not involve any further cellular divisions and lasts 18.6 days. Therefore, the total duration of spermatogenesis is 57 days in stallions. After leaving the testes, spermatozoa are transported through the epididymis, where final maturation occurs and sperm acquire fertilizing ability, a process that takes 9 to 14 days. Thus, sperm present in the ejaculate began being produced 66 to 71 days earlier, and sperm morphology is a reflection of events that occurred in the past 2 months that influenced spermatogenesis and sperm transport and maturation through the epididymis.

The mildest form of testicular degeneration produces no grossly detectable signs on the testes and is manifested exclusively by increased production of abnormal sperm. Elevated testicular temperature and endocrine disruption are probably the most common causes of mild testicular degeneration. Changes in sperm morphology are manifested in the ejaculate after an interval that varies according to the developmental stage of the germ cells at the time of the insult and the time required for the damaged cells to be released into the seminiferous tubules and transported through the epididymis. Although no similar research has been conducted in stallions, one experiment in bulls demonstrated that elevated testicular temperature and endocrine disruption produce similar changes in sperm morphology.[9] Different testicular germ cells have different sensitivity to elevated testicular temperature and endocrine disruption. Spermatids and spermatocytes are particularly sensitive, whereas spermatogonia and sperm in the epididymis are more resistant. If exposure to the insult is limited, a consistent sequence of appearance of sperm defects in the ejaculate is expected, whereas if exposure to the insult is prolonged, a variety of sperm defects may be present in the ejaculate at the same time. Semen quality improves as the spermatogonias that resisted to the insult restart producing normal sperm.

Normal spermatogenesis in scrotal mammals depends on maintenance of optimum testicular temperature 3-5°C below body temperature. Metabolic rate and oxygen demand increase as a result of elevated testicular temperature; however, the long and extremely coiled testicular artery limits the blood supply to the testes. Since blood flow in the testis does not increase at all, or not enough to match the increased metabolic rate of the heated tissue, testicular hypoxia develops with consequent detrimental effects on sperm production and quality.[10] Scrotal insulation in stallions for 24 to 48 hours resulted in increased production of abnormal sperm approximately 10 days after insulation, with a peak observed between 25 and 35 days after insulation. Preinsulation levels of normal sperm were only observed 50 to 75 days after insulation.[11,12] Causes of increase testicular temperature include increased whole body temperature (high ambient temperature, intensive exercise, fever), increased local temperature (scrotal trauma or dermatitis, orchitis, periorchitis, epididymitis), decreased local heat irradiation (hydrocele, scrotal edema, fat accumulation around the spermatic cords), and alteration of normal testicular mobility (tunic adhesions, inguinal and scrotal hernias). Scrotal suspensories that have become popular in show-jumping and trotting stallions have been demonstrated to result in 1-2°C increase in scrotal surface temperature above the normal increase in temperature during riding exercise.[13] Therefore, scrotal suspensories should be removed immediately after training or competition, or their use should be discouraged altogether.

Maintenance of adequate intratesticular testosterone concentration is essential for normal spermatogenesis. Endocrine disruption leading to decreased testosterone production results in increased production of abnormal sperm. Among the most common endocrine disruptors are cortisol, anabolic steroids, and progestagens. These hormones inhibit gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and ultimately testosterone production. The deleterious effects of cortisol on spermatogenesis have been demonstrated directly by treatment of bulls with corticosteroids.[9] Illnesses, extenuating exercise, fatigue, drastic changes in nutrition or management, show circuit competition, changes in social dominance, and long distance transport are some of the events that may result in stress with increased production of cortisol and abnormal sperm. Clearly, stress must persist for a period that is long enough for decreased testosterone concentration to affect semen quality. Repeated strenuous exercise for a period of 4 weeks resulted in increased circulating cortisol concentrations and increased production of abnormal sperm 3 weeks after the beginning of the exercise period in stallions. Curiously, testosterone concentration right after exercise was also increased, what was attributed to adrenal stress-induced production. The elevated cortisol concentrations probably had a more prolonged effect on testicular testosterone production that was not detected immediately after exercise. The percentage of normal sperm was similar to that observed before the exercise period only 3 to 4 weeks after the exercise routine had ceased.[14]

Testosterone and other anabolic steroids have been recommended as adjunct therapy to debilitated horses, but are also used in healthy horses to maximize muscular development and performance in halter and athletic animals. Not only does the administration of anabolic steroids to horses constitutes a “doping-offense” in many sports, but it also adversely affects stallion sperm production. Treatment of stallions with testosterone or anabolic steroids results in reduced LH secretion, depletion and atrophy of Leydig cells, decreased testicular steroidogenic enzymes, and reduced testosterone secretion, total scrotal width, sperm production, and percentage of normal sperm.[15-18] In one experiment, the percentage of normal sperm returned to pretreatment levels only 2 months after the last testosterone treatment.[16] In another study, the

percentage of sperm defects (proximal cytoplasmic droplets) was greater at 2 years, but not at 3 years of age, in colts treated with anabolic steroids from 7 to 12 months or from 12 to 24 months of age. Colts treated from 3 to 8 months with similar dose/regimen did not have increased abnormal sperm at 24 or 36 months of age.[18] It is apparent that, although the adverse effects of anabolic steroids do not seem to be irreversible, it can take several months for semen quality to return to normal after treatment with these drugs.

Progestagens are sometimes administered to stallions in attempts to suppress "aggressive" behavior (often just normal stallion-like behavior), but these treatments also interfere with sperm production in a manner very similar to anabolic steroids. Altrenogest administered to stallions resulted in decreased LH and testosterone secretion, total scrotal width, sperm production, and percentage of normal sperm. Although scrotal width and sperm production increased after cessation of altrenogest treatment or after initiation of GnRH treatments, neither sperm morphology had improved to pretreatment levels 100 days after cessation of treatment nor did GnRH treatments prevent the decrease in the percentage of normal sperm.[19] The author is not aware of studies evaluating the effects of a depot, long-acting formulation of medroxyporgesterone acetate (Depo-provera) on semen quality in stallions, but the adverse effects of this compound would be expected to be either similar or more pronounced than the short-acting altrenogest. Owners and trainers should be informed about the prolonged deleterious effects of progestagens on semen quality to weight the potential benefits of using these compounds for stallion behavior modification.

Although there is clear evidence that sperm production decreases during the nonbreeding season in stallions, results of studies regarding the effect of season on sperm morphology are controversial. In Europe, one study demonstrated that sperm abnormalities decreased during the breeding season,[20] another study described that the percentage of abnormal sperm increased during the breeding season,[21] whereas another study detected no difference in sperm morphology between the breeding and nonbreeding seasons.[22] Other reports from Europe indicated that season may have different effects on sperm morphology depending on the stallion's breed. One study reported that the percentage of normal sperm during the breeding season was lower during the summer when compared with spring and to the nonbreeding season (autumn and winter) in Warmblood stallions,[23] whereas the percentage of normal sperm was greater during the autumn and spring when compared with winter and summer in Franches-Montagnes stallions.[24] The differences in sperm morphology among seasons may be a result of differences in photoperiod, cold stress, and/or food quality. Regardless the cause, the effects of season on sperm morphology also probably involve endocrine changes related to central disturbance of gonadotropins and testosterone secretion.

Semen quality seems to increase after puberty and decrease with advanced age in stallions. The effects of age are probably related to factors such as inefficient spermatogenesis in colts and testicular degeneration due to aging in older stallions. In one study, the percentage of normal sperm increased from 33% at puberty (approximately 21 months of age) to 44% at 24 months of age.[25] In another study, colts less than 3 years old had the highest percentage of sperm with abnormal heads, proximal cytoplasmic droplets, and abnormal tails. There was a general trend for the percentages of sperm with abnormal heads and abnormal tails to decrease with age from 3 to 9 years of age.[26] Idiopathic testicular degeneration is a condition in which no underlying cause for the degeneration can be identified. Although this condition can affect young stallions, it is most often seen in middle-aged or older stallions. Idiopathic testicular degeneration comprises a heterogeneous group of problems that seems to be related to abnormal testicular steroidogenesis involving abnormal Sertoli cell function and inhibin secretion, rather than abnormalities of the hypothalamus–pituitary axis. Idiopathic testicular degeneration might produce detectable changes on testicular size and consistency, is progressive, and results in a steady decline in sperm production and semen quality.[27]

Some studies have described the effects of breed on sperm morphology. In the Netherlands, Warmblood, Welsh, and New Forest stallions evaluated at 3 years of age for breed registry seemed to have greater percentage of normal sperm (approximately 70%) than Friesian and Shetland stallions (approximately 55%).[28] Other authors also reported breed effects on sperm morphology, but the differences in history and management, the large differences in the number of stallions of each breed, the large within breed variation, and the lack of evaluation of interactions with age make it very difficult to ascertain whether the effects on sperm morphology could be attributed only to breed in these studies.[26,29] The bottom line is that individual variation seems much larger than the variation among breeds for the later to be a major concern to the veterinarian. Interestingly, however, a recent study in Shetland pony stallions has demonstrated that coefficients of inbreeding above 2% were associated with greater percentages of abnormal sperm. The percentage of live morphologically normal sperm decreased from 47.6% in stallions with inbreeding coefficient $<1\%$ to 32.6% in stallions with inbreeding coefficient $>12\%$.[30]

The safety of a few pharmacological compounds used in veterinary therapy for breeding stallions has been tested. The antiinflammatory drugs phenylbutazone and vedaprofen and the antihelminthic drugs cambendazole and ivermectin did not result in altered sperm morphology.[31-34] Treatment with antibiotics recommended for equine protozoal myeloencephalitis (trimethoprim-sulfamethoxazole and pyrimethamine), although resulting in transient changes in copulatory behavior suggestive of musculoskeletal stiffness across the back and possibly neurological deficits in the hind limbs and ejaculatory apparatus, did not interfere with normal sperm production.[35]

Anatomy of the Spermatozoon

The spermatozoon consists of a head, neck, and tail. The tail is the longest part of the spermatozoon and consists of midpiece, principal piece, and end piece (Fig. 1).[5,36-38] The plasma membrane, or plasmalemma, surrounds the spermatozoon in total and is more firmly attached to the caudal margin of the head, the annulus, and along the longitudinal columns of the principal piece. The length of the stallion spermatozoon is approximately 60 μm.[36]

The spermatozoon head is formed by the acrosome, the

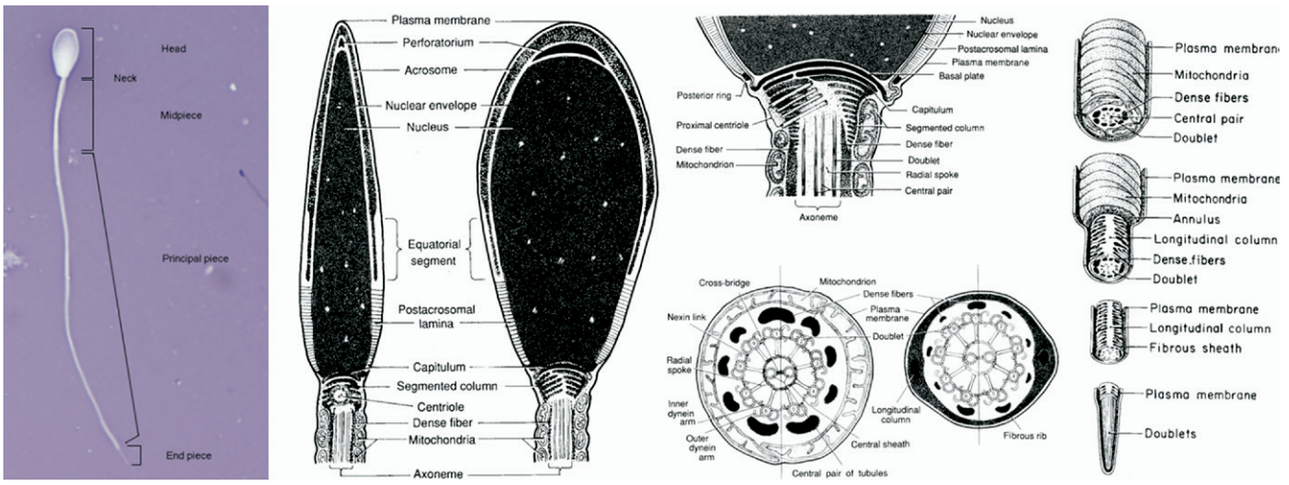

Figure 1 Anatomy of the spermatozoon. The spermatozoon consists of the head, neck, and tail and is entirely covered by the plasma membrane. The spermatozoon head is formed by the acrosome covering the anterior two-thirds of the nucleus, the postacrosomal lamina covering the remaining of the nucleus, and the nucleus enclosed within a double-layered membrane. Small nuclear vacuoles are observed through the condensed chromatin, and the perforatorium is observed between the acrosome and the anterior portion of the nucleus. Thickening of the nuclear membrane forms the basal plate at the base of the nucleus, which attaches the head to the capitulum of the neck. Segmented columns and the proximal centriole are also observed in the neck. The tail is divided into midpiece, principal piece, and end piece. The axoneme extends through the entire length of the tail and is formed by nine doublets of microtubules arranged around a central pair of microtubules. The doublets are interconnected by nexin links, and radial spokes connect them to the central pair of microtubules, which are connected with each other by a short cross bridge. Dynein arms project from one doublet toward the next doublet. Nine outer dense fibers surround the axoneme throughout the length of the midpiece and principal piece. The axoneme and dense fibers are surrounded by the mitochondrial helix in the midpiece and by a fibrous sheath formed by two longitudinal columns and circumferential ribs in the principal piece. The annulus demarcates the division between the midpiece and the principal piece. Axoneme doublets are the only structures observed in the end piece (Microphotograph: eosin-nigrosin stained smear. Diagrams from Amann RP, Graham JK. Spermatozoal function, in McKinnon AO, Voss JL (eds): Equine Reproduction. Philadelphia, PA, Lea & Febiger, 1993, pp 715-745, with permission from Blackwell Publishing). (Color version of figure is available online.)

postacrosomal lamina, and the nucleus (Fig. 1). The anterior two-thirds of the nucleus is overlaid by the acrosome, which is a specialized vesicle formed from a double-layered membrane which contains hydrolytic enzymes essential for spermatozoon penetration of the oocyte. The perforatorium is a cone-shaped accumulation of electron dense material between the acrosome and the anterior portion of the nucleus. The postacrosomal lamina is composed of very characteristic tight lamellae of high electron density and covers the caudal portion of the nucleus and caudal ring. The nucleus compromises most of the spermatozoon head and contains the genetic material in the form of highly condensed DNA. Small nuclear vacuoles of irregular contour without surrounding membranes are distributed at random through the condensed chromatin. These seem to be local defects in chromatin condensation and are typical for some mammalian sperm. The nucleus is contained by a double-layered nuclear envelope. The base of the nucleus terminates with the implantation fossa, where the outer layer of the double-layered nuclear envelope thickens to form the basal plate, which provides the attachment of the head to the capitulum of the neck. The border between the head and neck is clearly defined by a posterior ring and corresponds to the place of attachment of the plasmalemma with the nuclear envelope at the base of the head.

The stallion spermatozoon head is oval elongated with the anterior third being the widest part. The head is relatively flat and a longitudinal section has an approximately elliptical shape with a slightly thicker posterior end (Fig. 1). Reported means for dimensions of the stallion spermatozoon head include: 5.33 μm to 6.62 μm for length, 2.79 μm to 3.26 μm for maximum width, 0.43 to 0.52 for length/width ratio, 1.45 μm for width at the base, 13.76 μm to 15.64 μm for perimeter, and 11.43 μm^2 to 16.28 μm^2 for area. Despite the small number of stallions included in most experiments (5 to 20), all reports indicate a significant stallion effect on sperm head dimensions and coefficients of variation ranging from approximately 5% to 8%.[5,36,37,39-43] The variation in the shape of normal stallion sperm heads is considerable, ranging from somewhat thinner and elongated to shorter and broader forms (Fig. 2). The correct classification of sperm with extreme head shape morphology may be difficult, and the distinction of tapered and microcephalic sperm heads requires comparison among several sperm to establish what the "normal" sperm head shape for an individual stallion is. For example, the spermatozoon depicted in Fig. 2a could be classified as normal if most sperm head had similar shape or could be classified as abnormal head in a stallion in which the sperm head population uniformly resembles that of Fig. 2j, and vice versa.

There are suggestions that sperm head dimensions may be associated with fertility. Sperm head length, width, perimeter, and area were greater in subfertile stallions (<40% per cycle conception rate) than in fertile stallions (>60% per

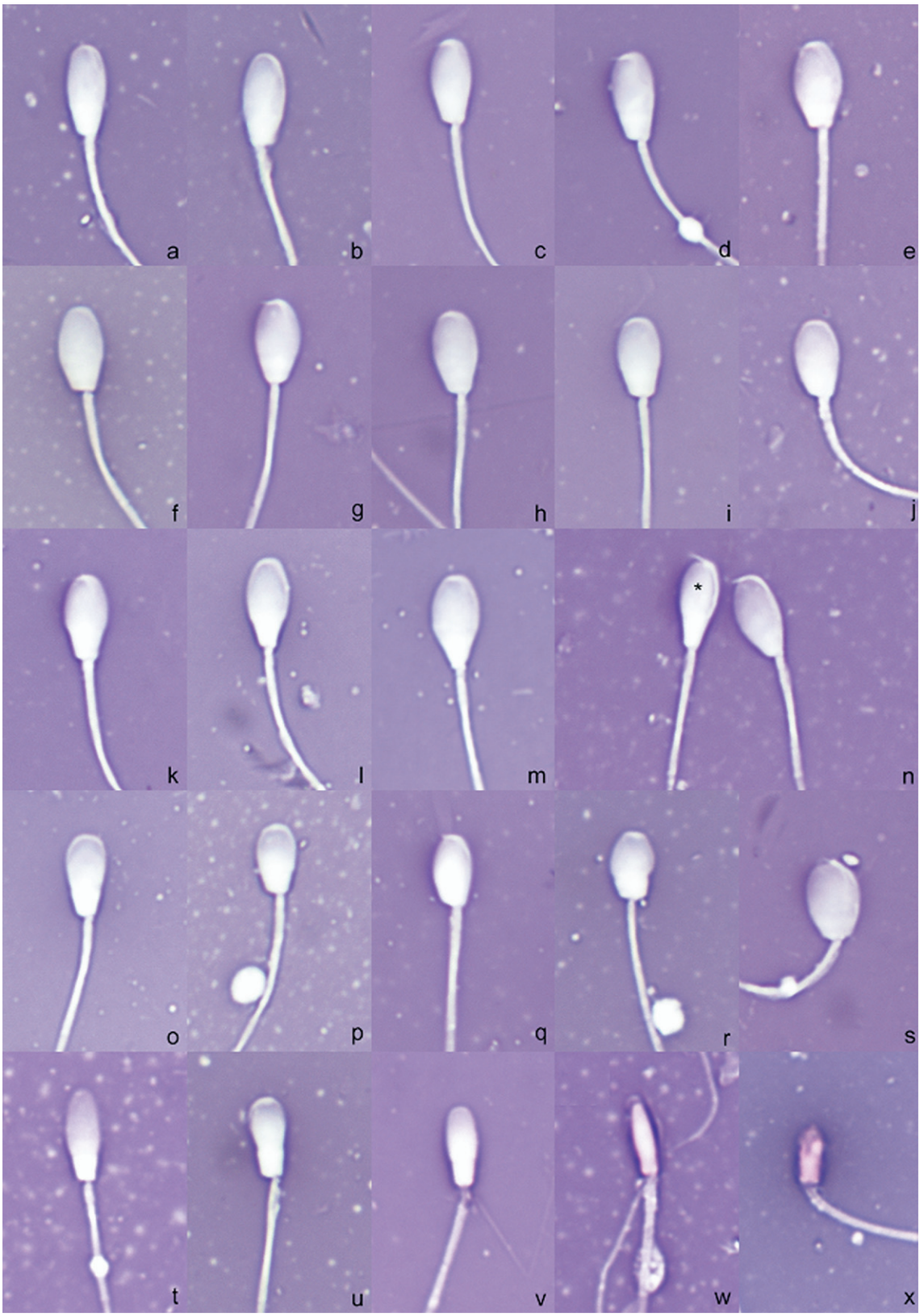

Figure 2 Sperm head morphology. There is considerable variation in the shape of normal stallion sperm heads, from somewhat thinner and elongated to shorter and broader forms (a-j). Comparison among several sperm to establish the "normal" sperm head shape for an individual stallion is necessary for correct classification of sperm with extreme head shapes. Although sperm heads with narrowness of the entire postacrosomal region are difficult to classify (k, l), pyriform (or narrow base) sperm heads are common defects of the sperm head shape (m, n; asterisk indicates pyriform head). Microcephalic sperm heads may have normal shape (o-q), rounder shape (r, s), tapered shape (t-v), or extremely abnormal shape (w, x). Eosin–nigrosin-stained smears. (Color version of figure is available online.)

cycle conception rate). Sperm in subfertile stallions also tended to be more tapered (lower length/width ratio) than in fertile stallions.[40,41] No significant differences in sample variation of any measurement were detected between subfertile and fertile stallions, indicating that the differences in dimensions were not related to a more heterogeneous sperm population in either group. The larger sperm heads found in subfertile stallions may reflect disturbances in spermatogenesis, particularly involving altered chromatin structure. However, it is important to note that subfertile stallions also had lower total sperm number and percentages of motile and normal sperm in the ejaculate than fertile stallions, which likely also influenced fertility.

The spermatozoon neck is a short linking segment between the head and the tail (Fig. 1). The neck is attached anteriorly to the basal plate and posteriorly to the outer dense fibers of the tail. The neck contains the connecting piece, the proximal centriole, and several small mitochondria. The connecting piece contains segmented columns and the capitulum. The segmented columns are formed from a fibrous protein, and each column is fused in the neck region to the anterior origin of one of the nine dense fibers. The two major segmented columns form the major portion of the capitulum, which is an enlarged head or ball which serves as the attachment with the basal plate of the head. In most stallion sperm, the implantation fossa and basal plate are eccentric in position with respect to the breadth of the cell, and thus sperm with abaxial tail are considered normal. The proximal centriole is located between the major segmented columns within the anterior end of the connecting piece and is positioned at a 45- to 60-degree angle to the tail axis. On the inner side of the striated columns, there are remnants of the posterior centriole connected with the microtubules of the axoneme. The place where two perpendicular mitochondria begin spiraling into the mitochondrial helix denotes the beginning of the midpiece.

The midpiece is formed by the axoneme surrounded by the outer dense fibers and the mitochondrial sheath (Fig. 1). It extends from the caudal end of the neck to the annulus and is 8 μm to 10.5 μm in length and 0.6 μm in diameter.[5,36,37] The axoneme contains microtubules doublets, which are the elements that contract to produce sperm tail movement. Axoneme microtubules extend from the neck region through the midpiece and principal piece into the end piece, where they terminate at slightly different sites. The axoneme is formed by nine doublets of microtubules forming a cylindrical bundle uniformly arranged around a central pair of microtubules. This arrangement of microtubules is referred to as 9 + 2 pattern. Each doublet is composed of a small cylindrical microtubule (subunit A) with an attached incomplete, C-shaped microtubule (subunit B). Both subunits are in contact with each other, and the subunit A of the microtubule doublet has two short, longitudinal dynein arms projecting toward the next doublet. The doublets are interconnected by nexin links and a series of nine radial spokes extend from the central pair to the doublets. The two central-pair microtubules are also connected with each other by a short cross bridge.

Each microtubule doublet of the axoneme is surrounded by one of nine outer dense fibers. These fibers have a petal-like shape and a tough, keratin-like fibrous structure. They extend from their origin in apposition to segmented columns in the neck of the spermatozoon through the length of the midpiece and most of the principal piece. All fibers are thickest in the proximal part of the midpiece and progressively taper away toward the end of the principal piece; they are not present in the end piece. The outer dense fibers are surrounded by mitochondria arranged end to end in a continuous double spiral. Approximately 60 mitochondrial spirals are observed in stallion sperm. Mitochondria are the membranous organelles that produce most of the energy necessary for spermatozoon motility. At the caudal end of the mitochondrial sheath is the annulus (or Jensen's ring) that consists of closely packed circumferentially oriented filamentous subunits and lies between the most caudal gyrus of mitochondria and the anterior end of the fibrous sheath of the principal piece. It demarcates the end of the midpiece and is a point where the plasma membrane is firmly attached.

The principal piece is approximately 30 μm to 44 μm[5,36] and is the longest segment of the tail. The axoneme and dense fibers of the midpiece continue through the principal piece, but the dense fibers become narrower and terminate at different levels in the distal principal piece. The unique feature of the principal piece is its enclosure by a fibrous sheath of proteinaceous material that is made of two longitudinal columns (dorsal and ventral columns) and circumferentially oriented connecting ribs halfway around the tail (Fig. 1). The dense fibers and fibrous sheath do not contract, but provide the structural support and flexibility essential for effective translation of the sliding motion of the doublets of the axoneme into tail movements of definitive flexure and amplitude. The fibrous sheath ends abruptly a few micrometers from the tip of the tail where the principal piece merges into the end piece. The end piece is the short terminal segment of the tail containing only the axoneme (Fig. 1).

Abnormal Morphology of Stallion Sperm

The first step in evaluating stallion sperm morphology is to remove the gel from the ejaculate, because gel interferes with the ability to visualize the sperm. Another important point is that sperm morphology should always be evaluated under 1000× or greater magnification. Sperm morphology can be evaluated by examining wet mount preparations of unstained samples fixed (1:4 ratio) in buffered-formol saline (see composition[44]) under phase-contrast microscopy. This method allows excellent visualization of sperm defects, but the requirement of 1000× phase-contrast objective limits its use to well-equipped laboratories. When using this method, it is important to allow some time after the preparation of the wet mount for sperm to settle flat on the slide to allow proper examination. Sperm morphology can also be evaluated by examining stained smears under bright field-microscopy, and several staining methods have been used for this purpose (eg, India ink, William's, Karras, Spermac, Diff-Quick, eosine-aniline blue[28]). Some authors have indicated the possibility that staining sperm might induce acrosome, head, or tail abnormalities, but no direct comparison between fixed wet mounts and dry stained samples have been reported in stallions. As a matter of fact, after a discussion about methods for evaluating sperm morphology in dogs and laboratory ani-

mals, a group of 15 renowned andrologists was hesitant in recommending one method over another due to the lack of scientific evidence to support the superiority of any given method.[45]

In North America the most commonly used stain for evaluation of sperm morphology is the eosin–nigrosin (http://www.therio.org/storeindex.cfm). Eosin is a supravital stain because it does not penetrate cells with intact membranes. Therefore, unstained sperm have intact membranes (live), and those staining red (even partially) have disrupted membranes (dead). Nigrosin provides a purple background that allows visualization of unstained sperm. Stained slides are prepared by placing roughly equally sized droplets (3 to 5 mm) of stain (first to avoid contamination of the stain bottle with sperm) and semen on a prewarmed glass slide. Stain and semen are mixed together, and the mixture is smeared using a wooden stick or another slide. Adequate contrast is important for proper evaluation. The background should not be so light that unstained sperm are difficult to see, but should not be so thick that it cracks. Smearing the mixture with jerking movements produces several bands of different contrast on the smear, allowing the evaluator to choose the area with the best contrast for examination. The slide should be placed over a warming tray and gently blown for quickly drying because the stain is hypotonic in relation to semen and quick drying prevents artifacts from hyposmotic shock. Regardless the method used to prepare the specimen, at least 100 sperm should be examined and classified. Detached heads, but not headless tails, are counted. Both unstained (live) and stained (dead) spermatozoa are examined and classified when evaluating eosin–nigrosin-stained smears; a separate count should be done to determine the percentages of live and dead sperm.

Some authors have used a sperm morphology evaluation system in which defects are prioritized (usually the most proximal defect) so that only one is recorded per spermatozoon. This system is based on the assumption that certain defects are more important or more deleterious to fertility than others, which is an assumption not necessarily based on scientific evidence. Furthermore, it is not possible to record the distribution of sperm defects in the ejaculate and to track changes in specific defects, so that the use of this system is discouraged. It is preferred to enumerate all the defects on a single spermatozoon to track the changes in the patterns and associations of specific sperm defects, which provides the veterinarian a better overall picture of the condition and greatly enhances the ability to determine breeding soundness.[46,47] To enumerate multiple defects simultaneously, the evaluator presses the keys for the defects simultaneously on a cell counter so that this only advances the total cell count one number. Using this system, the percentages of sperm defects when added to the percentage of normal sperm will not total 100%. Some authors have also advocated reporting the percentages of sperm with single and with multiple defects, since different defects when found together represent more severe disturbances in spermatogenesis and could influence the prognosis.[47]

Classification systems for sperm defects proposed for bulls have been adapted by some authors for stallions.[44,48] In one classification system in which sperm defects are classified according to their origin, primary sperm defects are assumed to have occurred during spermatogenesis, and secondary defects are assumed to have occurred during the transit through the excurrent tract. The major limitations of this classification system are the unknown origin of some sperm defects and the fact that primary defects are not necessarily more deleterious to fertility than secondary defects, a common misinterpretation of this system. Another system classifies sperm defects into major and minor defects according to the perceived effects on fertility. Obviously, this classification system can only be used when there is a considerable body of data describing the effects of specific sperm defects on fertility, which is not the case in stallions. Moreover, to remain meaningful, this classification system would have to be revised periodically to incorporate the knowledge gained with new research and this has not occurred even in bulls. According to Barth and Oko,[48] an additional disadvantage of classification systems like such is that veterinarians tend to evaluate the spermiogram as a simple case of mathematics, listing the percentages of each defect, grouping defects in appropriate categories, and checking a chart to determine whether the animal should be considered a satisfactory prospective breeder. If these systems are not used, veterinarians would perhaps give more serious consideration to the significance of the various sperm defects observed and consider those in the light of other findings of the breeding soundness evaluation.

Several other classification systems have been reported in stallions and the nomenclature used for specific defects varies widely.[22,44,49] Although the adoption of a standard classification system and standard nomenclature for specific defects would have enormous benefits for both basic and applied research and ultimately for the ability of veterinarians to interpret the findings of the breeding soundness evaluation, this has not happened in any species. The present Society for Theriogenology forms for stallion breeding soundness evaluation have the following categories listed in the differential spermiogram: normal sperm, abnormal acrosomal regions/heads, detached head, proximal droplets, distal droplets, abnormal midpieces, and bent/coiled tails. The presence of other cells (round germ cells, WBC, RBC, etc.) should also be indicated.[46] Ideally, the specific defects of different sperm regions should also be enumerated. Acrosome defects include knobbed, roughed, and detached acrosomes. Head defects include microcephalic (small, underdeveloped, or dwarf), macrocephalic (large or giant), pyriform (narrow at the base), tapered (narrow), other shape defects (those usually also micro or macrocephalic), nuclear vacuoles (pouches or craters), and multiple heads. Midpiece defects include midpiece reflex (simple bent or folded midpiece), segmental aplasia of the mitochondrial sheath, fractured, swollen (thick, pseudodroplet), roughed (corkscrew), swollen/roughed/broken, (Dag-like), disrupted sheet (filamentous), duplicated and stump tail. Bent or coiled tails refer to those sperm in which both the midpiece and the principal piece are bent or coiled, or the distal part of the principal piece is coiled. The percentage of normal and abnormal detached heads (tailless or separated heads) could be recorded separately, and teratoids ideally should be classified as a complete separate category.

The pathogenesis and effects on fertility of specific sperm defects have been more extensively studied in bulls, and this information will be herein presented where pertinent. The most common defect of the acrosome is the knobbed acro-

some, which consists of an excess of acrosomal matrix and folding of the acrosome over the apex of the sperm head. Membranous vesicles containing granular or membranous inclusions are commonly entrapped in the acrosomal matrix.[48,50] The appearance of this defect on light microscopy varies from bead-like thickening on the sperm head apex (sometimes protruding from the head ridge) to indentation and flattening of the apex (Fig. 3a-e). The knobbed acrosome can be caused by environmental factors (eg, increased testicular temperature, stress, toxins), but can also be of genetic origin. In bulls, genetic knobbed acrosomes are caused by an autosomal sex-linked recessive gene, whereas in boars, the defect has been associated with both dominant and sex-linked recessive genes.[51] Genetically affected animals consistently produce great percentages of affected sperm without significant changes in other sperm defects.[48,51] Knobbed acrosomes of genetic origin have not been reported in stallions. In bulls, knobbed acrosomes develop during spermiogenesis.[48]

The overall incidence of acrosome defects detected by light microscopy seems to be low in stallions,[52] but might be high in some individuals. Hurtgen and Johnson[50] reported data from seven stallions that were identified as having a high percentage of sperm with acrosome defects. The percentage of motile sperm, morphologically normal sperm, and acrosome defects were 30% to 65%, 20% to 49%, and 38% to 53%, respectively. For stallions collected multiple times (2 to 16 times), the percentage of abnormal acrosomes varied as much as 47% (27 to 74%). Acrosomal abnormalities occurred more frequently in conjunction with other sperm abnormalities (40% in otherwise normal sperm versus 64% in abnormal sperm), suggesting impaired spermatogenesis. Folding of the acrosome and flattening of the apical ridge

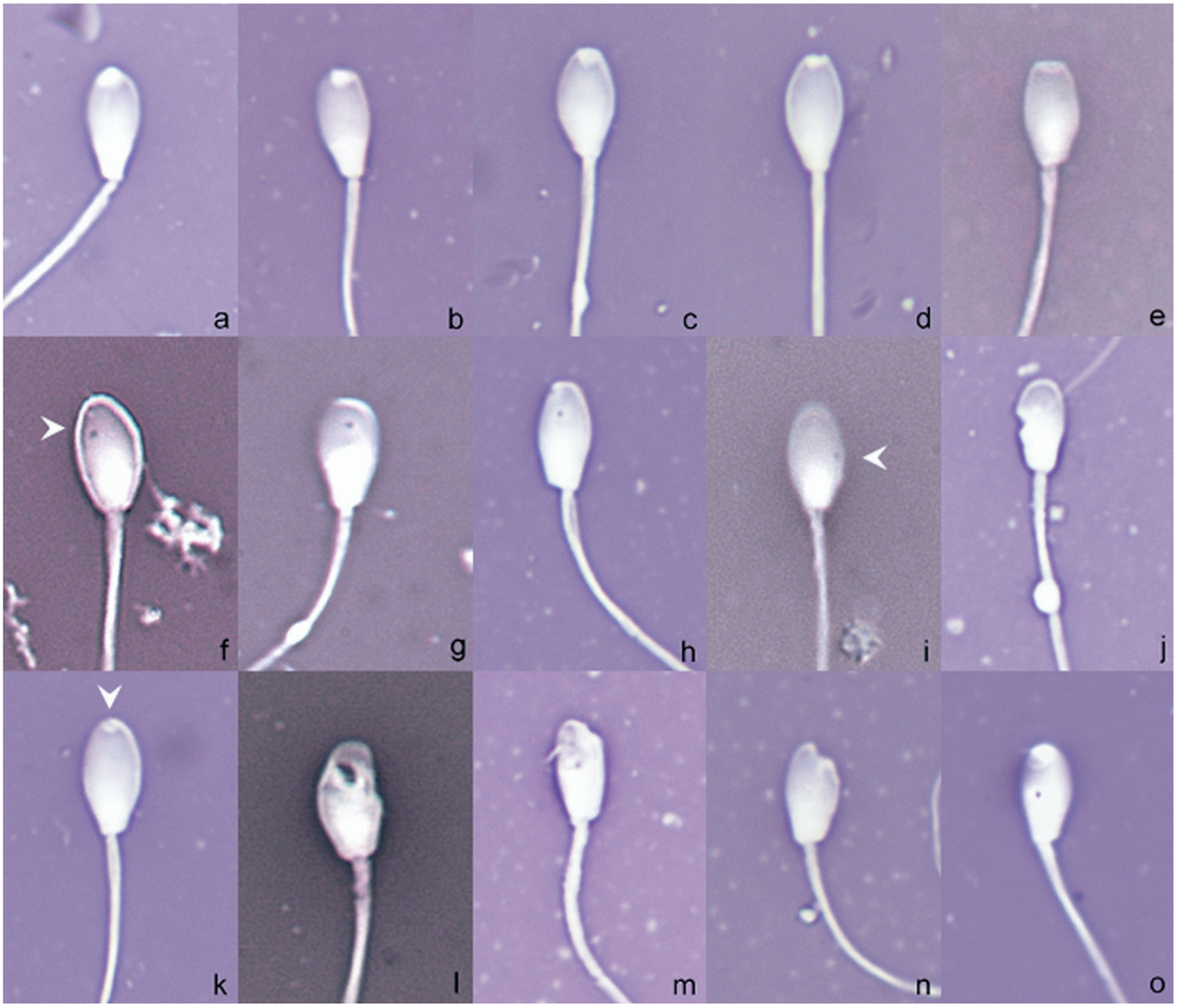

Figure 3 Sperm head morphology. The most common acrosome defect is the knobbed acrosome, which appearance varies from bead-like thickening on the sperm head apex sometimes protruding from the head ridge (a-c) to indentation (d) and flattening of the apex (e). Nuclear vacuoles appear as dark dots and can be observed anywhere on the sperm head (f-i; arrowheads point to vacuoles in f and i). Vacuoles might be large and cause deformity of the sperm head shape (j, l-n). Vacuoles located on the apex of the head might also involve abnormalities of the acrosome (k-n; arrowhead points to vacuole in k), and sometimes both defects are clearly observed concurrently (o). Eosin–nigrosin-stained smears. (Color version of figure is available online.)

were the most common forms, but roughing of the acrosome, bead-like protrusion from the apical ridge, and vacuole within the acrosome were also observed. Recent studies using electronic microscopy have revealed that the actual incidence of acrosome defects might be much higher than that observed with light microscopy. Prematurely reacted acrosomes, undulant acrosomes, acrosomes separated from the nucleus, invagination of the acrosomal membrane into the nucleus, aplasia or rarefaction and dissolution of the acrosome, vesiculations and amorphous inclusions are some of the acrosome defects detected by electronic microscopy.[53-55]

High percentage of knobbed acrosomes in the ejaculate of genetically affected bulls results in virtually sterility.[48] In vitro studies suggested that sperm with knobbed acrosomes have altered plasmalemma function that predisposes to premature sperm capacitation and spontaneous acrosome reaction.[56] Sperm with knobbed acrosomes are unable to bind and penetrate the zona pellucida. Moreover, other genetic defects in otherwise morphologically normal sperm capable of fertilizing the oocytes probably contributed to the impaired embryonic development observed in vitro after the use of semen from bulls producing genetic knobbed acrosomes.[57,58] In stallions, mating three stallions producing 38% to 50% abnormal acrosomes to a limited number of mares resulted in per cycle pregnancy rates ranging from 12.5% to 47%. Surprisingly, however, another stallion producing approximately 50% abnormal acrosomes had 100% per cycle pregnancy rate after breeding eight mares.[50] A recent study reported that alterations involving acrosome reaction (especially after induced reaction) evaluated by electron microscopy was likely associated with idiopathic infertility in five stallions.[54] It seems that alternative methods for evaluation of the acrosome (electron microscopy, fluorescent probes) would be particularly useful in stallions.

The incidence of sperm head defects is relatively high and these are usually either the most or second most prevalent defects in the ejaculate.[26,49,52,59] The head shape is dictated primarily by the nucleus shape, which is in turn determined by extrinsic forces from the Sertoli cell, by the caudal manchette of the spermatid, or by intrinsic factors that affect nuclear chromatin condensation.[48] Any of these factors could be involved in the production of pyriform (Fig. 2k-n) and tapered sperm heads. In bulls these defects seem to develop during spermiogenesis not only when visible nuclear flattening and elongation of spermatids occur, but also when morphological changes in the nuclear shape are still not apparent.[9,60] Microcephalic (Fig. 2o-x) and macrocephalic sperm are probably the consequence of insults to primary and secondary spermatocytes that then have an uneven distribution of nuclear chromatin content after abnormal cell division.[48,60] Nuclear vacuoles in stallions might be present with or without invagination of the nuclear membrane into the nucleus.[53,55] Nuclear vacuoles appear as dark dots in eosin–nigrosin-stained smears and can be observed anywhere on the sperm head (Fig. 3f-j). In the author's experience, this is the most difficult defect to observe and careful adjusting the micrometer focus up and down during the examination facilitates the detection of this abnormality. Vacuoles located on the apex of the head might also involve abnormalities of the acrosome, and sometimes both defects are clearly observed concurrently (Fig. 3k-o). Nuclear vacuoles form during spermiogenesis in bulls, and there are suggestions of genetic predisposition to develop this kind of defect.[48,60]

Studies in bulls demonstrated that transport of sperm with tapered and pyriform heads was impaired (perhaps due to hydrodynamic alterations) and these sperm were selectively "filtered" throughout the female genital tract, so that only a small proportion of inseminated sperm with these defects were found as accessory sperm.[61] In vitro studies indicated that sperm with tapered and pyriform heads had reduced ability to bind the zona pellucida, but that the capacity to penetrate the zona and fecundate the oocytes after binding was unaffected. However, defective sperm seemed unable to sustain normal embryonic development after fecundation.[62] Similar studies demonstrated that sperm with nuclear vacuoles have reduced ability to bind and penetrate the zona pellucida, and that fertilization may either be compromised due to abnormal chromatin decondensation or normal with normal embryonic development al least until the blastocyst stage.[63,64] In stallions, Jasko and coworkers[59] observed a negative correlation between the percentage of sperm head defects and fertility and reported that, among sperm morphological categories, the percentage of head defects accounted for the largest proportion of variation in per cycle pregnancy rates. Love and coworkers[52] also observed an association between sperm head defects and fertility and estimated that a 1% increase in the percentage of head defects resulted in a 0.67% reduction in per cycle pregnancy rates. Held and coworkers[65] reported the case of a 9-year-old Arabian stallion used to breed an undetermined number of mares during 3 years without producing any pregnancies that had 92% abnormal sperm with 75% head defects, 57% of which with single or multiple nuclear vacuoles.

A common midpiece defect is the distal midpiece reflex (DMR), which on light microscopy appears as a bend in the distal region of the midpiece in the shape of the letter J. A distal cytoplasmic droplet is nearly always entrapped in the bend (Fig. 4a and b). In bulls, DMR develops in response to environmental insults as sperm migrate to the distal half of the epididymal tail, probably in association with altered ion concentrations.[9,48,60] Double bends of the midpiece usually accompany coiling of the principal piece with retention of cytoplasmic material (Fig. 4c-e). The difference between the DMR or bent/coiled tails and the Dag-like defect is that, in the former, the midpiece is smooth and complete, whereas in the latter, the bending and coiling involving the midpiece or the entire tail is associated with rough, incomplete mitochondrial sheet usually accompanied by fractures and shattering of the axonemal fibers (Fig. 4f-j). The Dag defect was named after a Jersey bull in which the defect was first identified and was later identified to be caused by a recessive gene.[48,51] Genetically affected bulls produce a large percentage of sperm with the defect and have extremely low fertility or sterility, but a small percentage of a similar defect (hence Dag-like) can sometimes be observed in association with other defects in cases of disrupted spermatogenesis. In stallions, Hellander and coworkers[66] reported the case of an 8-year-old Standardbred stallion with poor fertility that presented approximately 85% abnormal sperm tails (midpieces and principal piece defects) during 3 months of evaluation. Examination of sperm ultrastructure revealed disarranged mitochondrial sheaths, missing microtubules in the axon-

Figure 4 Sperm tail morphology. The distal midpiece reflex is a common defect that appears as a bend in the distal region of the midpiece in the shape of the letter J, usually with a distal cytoplasmic droplet entrapped in the bend (a, b). Double bends or coiling of the midpiece usually accompanies coiling of the principal piece with retention of cytoplasmic material (c-e). The Dag-like defect also involves bending and/or coiling of the midpiece or the entire tail, but is associated with rough, incomplete mitochondrial sheet and fractures and shattering of the axonemal fibers (f-j). Simple bend (k) or coiling with retained cytoplasm (l, m), and less commonly aplasia (n) or abnormal development (o) are some of the defects involving only the principal piece. Eosin–nigrosin-stained smears. (Color version of figure is available online.)

eme, missing and disarranged dense fibers, and microtubules present outside the fibrous sheath. Sperm motility was extremely poor (1% to 10%), and per cycle pregnancy rate was 24% in 32 mares artificially inseminated with approximately 3.6 billion sperm (237 million motile sperm). This defect was similar to the Dag-defect reported in bulls and resulted in subfertility. Simple bend or coiling (usually with retained cytoplasm), and less commonly aplasia or abnormal development are some of the defects involving only the principal piece (Fig. 4k-o).

Segmental aplasia of the mitochondrial sheet might be observed in a low percentage of sperm in varying degrees; some sperm lack a small part of the sheet, whereas others seem to miss the mitochondrial sheet completely (Fig. 5a and b). Segmental aplasia of the mitochondrial sheet creates a point of structural weakness that is predisposed to fracture when the sperm acquires motility. The fracture seems to occur more commonly in the region of the annulus but can occur in a more proximal region or in the middle of the midpiece (Fig. 5c-e). Pseudodroplet and corkscrew defects are rare midpiece defects described in bulls that are characterized by swollen or roughed midpieces, respectively, and that consist in abnormalities of the mitochondrial sheet.[48] Although specific characterization of these defects has not been reported in stallions, forms similar to that observed in bulls are sometimes encountered (Figs. 5f-h). Chenoweth and coworkers[67] reported the case of a 5-year-old subfertile stallion that produced a high percentage of sperm with a thick, rough midpiece that was overlaid by several layers of mitochondria of different shape, size, and orientation to the long axis of the sperm. Although this defect resembled the corkscrew defect reported in bulls, similarities with the Dag defect were also observed in the sperm from this stallion (ie, missing microtubules in the axoneme). Disruption of the midpiece and protrusion of axonemal fibers are occasionally observed (Fig. 5i and j).

One defect involving midpiece swelling that seems to be particular of stallions and that might be associated with some of the defects that resemble pseudodroplets, corkscrew, and even cytoplasmic droplets is the microtubular mass defect. Heath and coworkers[68] reported data from seven Standardbred stallions in which microtubular masses were observed on the neck or midpiece region and occasionally on the head. On evaluation by light microscopy, the percentage of normal sperm varied considerably among stallions and even within stallions over time. Microtubular masses consisted of tortuous arrays of microtubules of variable length, unbranched, and 18 to 19 nm in cross-sectional diameter (about 80% of the diameter of axonemal microtubules). Three of the seven stallions had the same sire, indicating a possible genetic cause of microtubular mass defect in stallions. All the stallions produced pregnancies, and there was not a clear adverse effect on fertility. Alvarenga and Alvarenga[69] reported the case of a 5-year-old sterile Arabian stallion that produced sperm with normal tail beat frequency, but that failed to move forward even after addition of semen extenders. As judged by light microscopy, approximately 62% of sperm were normal; however, examination of sperm ultrastructure revealed randomly arranged microtubules extruding from the sides of the neck in approximately 90% of sperm with no apparent alteration of the axoneme structure.

Duplication of the tail (Fig. 6a and b) is an uncommon defect that is associated with duplication of the implantation fossa and replication of the distal centriole. Sperm with multiple heads and tails (Fig. 6c and d) might have normal head structure with normal DNA content, but abnormalities of nuclear shape and abnormal DNA condensation in one or more heads might also be observed. The heads are usually completely separated, and in the tail, independent axial filament complexes are kept together by a common mitochondrial or fibrous sheet. Abnormalities of the tail, like disintegration of the axial filament complex and disorganization of the mitochondrial sheet, might occur. These sperm originate from multinucleated spermatids and/or as the result of incomplete cell dissociation during spermatogenic divisions.[70] The incidence of specific midpiece and tail defects and their effects on fertility in horses are difficult to ascertain because those are seldom reported separately. Some authors have observed a negative correlation between midpiece/tail defects and per cycle pregnancy rates.[55,59] Love and coworkers[52] observed no correlation of midpiece bents and fractures with fertility; however, these authors estimated that a 1% increase in the percentage of other midpiece abnormalities resulted in a 2.9% reduction in per cycle pregnancy rates, whereas a 1% increase in the percentage of coiled tails resulted in a 3.9% reduction in per cycle pregnancy rates.

Sperm cytoplasmic droplets are normal remnants of the spermatid residual cytoplasm that remain attached to the neck region of sperm after release into the seminiferous tubules. Virtually all sperm have cytoplasmic droplets in this proximal location as they move into the head of the epididymis. During the maturation process, along the transit through the body of the epididymis, the droplet moves from this proximal neck position to the distal portion of the midpiece adjacent to the annulus. In bulls, approximately 35% of sperm shed the distal droplet in the tail of the epididymis, but the majority of sperm only shed the distal droplet after mixed with secretions from accessory sex glands.[48] Therefore, proximal cytoplasmic droplets are prevalent in the head of the epididymis, and distal droplets are prevalent in the tail of the epididymis, but cytoplasmic droplets on ejaculated sperm are abnormal. Cytoplasmic droplets contain vesicles, tubules, and vacuoles and appear as small spherical masses (Fig. 5k and n). The location, size, and contour differentiate cytoplasmic droplets from other midpiece defects (Fig. 5f, g, l, m, and o). It is common to observe shed cytoplasmic droplets on semen samples and those should not be confused with other cell types like round germ cells and WBC, which are much larger than droplets and usually at least 1.5 times larger than sperm heads. Sperm cytoplasmic droplets are often the most prevalent defect in the ejaculate, especially in young peripubertal stallions.[26,46,49,52,59]

Although proximal cytoplasmic droplets may result from impaired epididymal function, research in bulls indicated that cytoplasmic droplets may result from insults to spermatids in any stage of spermiogenesis and even to spermatocytes.[9,60] Proximal cytoplasmic droplets have severe adverse effects on fertility in bulls, and levels as low as 10% may be associated with lowered fertility.[48] In vitro studies demonstrated that sperm with proximal cytoplasmic droplets are not capable of binding and penetrating the zona pellucida. Moreover, other genetic defects in otherwise morphologi-

Figure 5 Sperm tail morphology. Segmental aplasia of the mitochondrial sheet might be observed in varying degrees from small segments in some sperm (arrowhead in a) to the apparent complete lack of mitochondria in others (b). Segmental aplasia of the mitochondrial sheet creates points of structural weakness that are predisposed to fractures (c-e). Swollen (f, g) and roughed (h) midpieces might involve abnormalities of the mitochondrial sheet or accumulation of microtubular masses. Disruption of the midpiece and protrusion of axonemal fibers are occasionally observed (i, j). Cytoplasmic droplets appear as small spherical masses attached to the neck region (k; proximal droplet) or to the distal portion of the midpiece adjacent to the annulus (n; distal droplet). The location, size, and contour differentiate cytoplasmic droplets from other midpiece defects, like pseudodroplets, residual cytoplasm (l), and microtubular mass defects (possibly the defect represented in m and the proximal swelling in o). Eosin–nigrosin-stained smears. (Color version of figure is available online.)

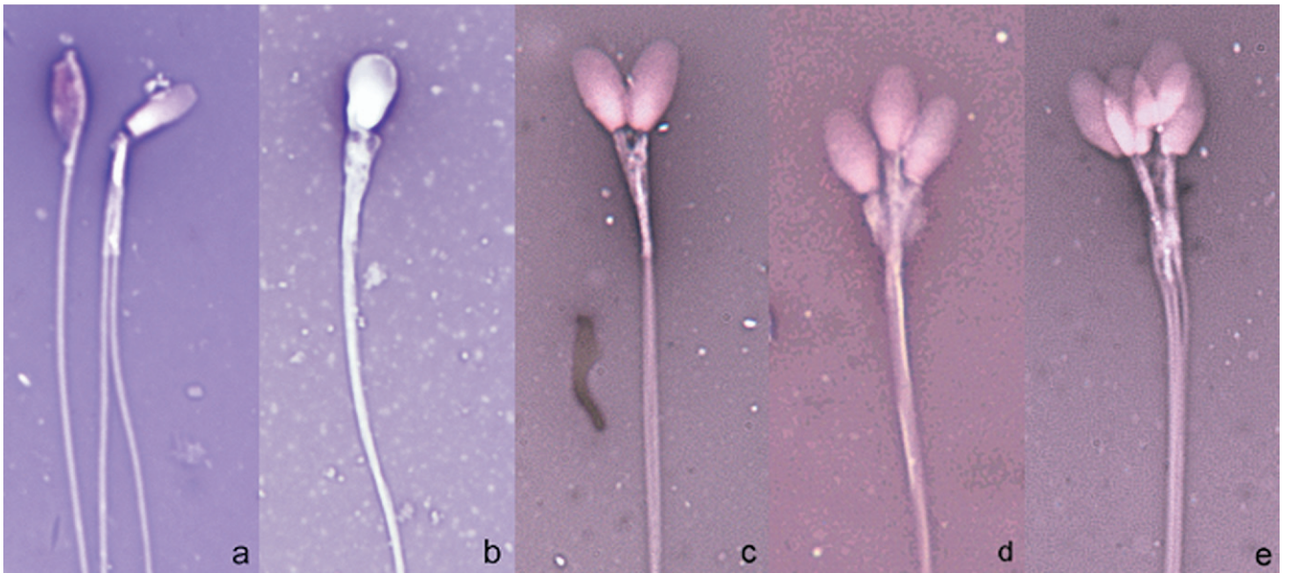

Figure 6 Duplication of the tail (a, b) and sperm with multiple heads and tails (c-e) are occasionally observed. Eosin–nigrosin-stained smears. (Color version of figure is available online.)

cally normal sperm capable of fertilizing oocytes probably contributed to the impaired embryonic development observed in vitro after the use of semen from bulls producing a large percentage of sperm with proximal droplets.[71,72] On the other hand, breeding trials in cattle demonstrated that distal cytoplasmic droplets do not affect fertility and these have been disregarded as a defect (A.D. Barth, personal communication). In stallions, Jasko and coworkers[59] observed that the negative correlation between the percentage of proximal cytoplasmic droplets with per cycle pregnancy rates was three times greater than the correlation with distal droplets, and only the former variable accounted for a significant percentage of variation in fertility. Persch and coworkers[55] indicated a negative correlation between the percentage of cytoplasmic droplets and per cycle pregnancy rates, but did not differentiate proximal from distal droplets in their report. In another study, however, the percentage of proximal cytoplasmic droplets was not associated with fertility in stallions.[52]

Detached sperm heads are commonly observed in low percentages (<5%) in the ejaculate,[26,49,52,59] but might be present in very high numbers in cases of sperm accumulation in the excurrent tract.[46] Detached heads might result from abnormal spermiogenesis or from sperm senescence in the tract. Obviously, abnormal detached heads indicate more severe disturbance of spermatogenesis than normal detached heads. In cases of sperm accumulation, detached sperm heads and most of the complete sperm have disrupted membranes and are stained with eosin (Fig. 7a). Love and coworkers[52] estimated that a 1% increase in the percentage of detached heads resulted in a 2.6% reduction in per cycle pregnancy rates in stallions. The tail stump is a rare genetic defect in bulls that consists in an anomaly of development of

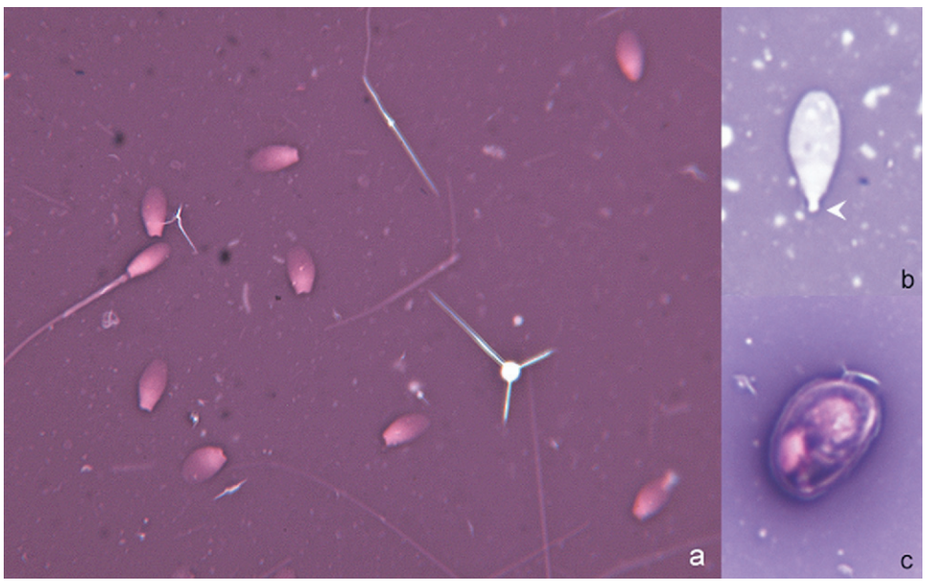

Figure 7 A large proportion of eosin-stained detached sperm heads is usually observed in cases of sperm accumulation (a). Careful examination of apparent detached heads might reveal the existence of a tail stump (arrowhead in b). Teratoids are severely deformed cells barely recognized as sperm (c); attention should be paid not to confuse those with debris or other cells. Eosin–nigrosin-stained smears. (Color version of figure is available online.)

the distal centriole and affects 80% to 100% of sperm causing sterility.[48,51] This defect might occasionally be observed in a low percentage of sperm in stallions. On light microscopy, the initial impression may be of a detached head, but close examination reveals that the tail is replaced by a small stump (Fig. 7b). The term teratoid has been used to describe sperm that are so severely deformed that the cell is barely recognized as sperm (Fig. 7c). In most cases, the head has abnormal shape and the tail is tightly coiled around or over the head; these invariably take up eosin, indicating disruption of the plasmalemma. Attention should be paid not to confuse those with debris or other cells. A low percentage of teratoids may be occasionally observed in normal bulls and stallions, but a genetic cause in bulls[48] and testicular degeneration in rams (personal observation) might be associated with high percentages of this defect.

Other cells that might be found in the ejaculate include blood cells, round germ cells, and Sertoli cell mantles. Proper classification of these cells requires the use of different staining techniques that could include Giemsa or Diff-Quick. The use of a thick section of fixed sperm pellets stained with toluidine blue is also an excellent way to differentiate other cells in the ejaculate.[73] Blood cells (WBC and RBC) can originate anywhere in the reproductive tract, but are very rarely of testicular origin; careful examination of the stallion is necessary to identify the source. These cells might cause noticeable change in the color of the ejaculate (especially RBC) and present characteristic morphology after stained by Diff-Quick, ie, RBC are anucleated, round, pale gray cells, whereas WBC are larger cells (at least 1.5 times the size of the sperm head) with round or irregular contour, basophilic cytoplasm, and eosinophilic multilobulated nucleus. Round germ cells are occasionally observed in small numbers in stallions. Most round germ cells in semen are spermatids (many of which binucleate) and some are secondary or even primary spermatocytes.[73,74] These cells are approximately twice the size of sperm heads, might have intact or disrupted membranes, and show round nuclei when stained with Diff-Quick (Fig. 8a and b). Increased percentages of round germ cells are observed in peripubertal stallions and in cases of testicular degeneration when germ cells are prematurely shed in the seminiferous tubule lumen.[46,74] The fact that increased percentage or round germ cells constitute an indication of testicular degeneration is demonstrated by the estimation that a 1% increase in the percentage of these cells in the ejaculate resulted in a 21% reduction in per cycle pregnancy rates.[52] Increasing presence of Sertoli cell mantles containing degenerating germ cells have been reported in the semen of stallions with deteriorating, likely irreversible testicular degeneration and impaired fertility. Sertoli cell mantles might resemble sperm agglutination on light microscopy and require electron microscopy evaluation for differentiation.[53]

Conclusion

There is a wide variation in sperm morphology among breeding stallions,[26,49,52,59] but in general, the average stallion has approximately 50% morphologically normal sperm.[46] Sperm morphology can vary considerably during the breeding season, and routine evaluations (every 2 to 4 weeks) should be performed to determine sperm morphology characteristics of a particular stallion.[52] More than 30% sperm head defects, >25% proximal cytoplasmic droplets, or <40% normal sperm are reasons for concern.[1,46] In the author's opinion, the presence of round germ cells in the ejaculate is always reason for concern. In North America, the breeding soundness evaluation guidelines from the Society for Theriogenology were developed with the intent to select stallions that could render at least 75% of 40 or more mares pregnant when bred naturally or 120 mares when bred artificially. According to these guidelines, a satisfactory prospective breeder should produce a minimum of 1 billion morphologically normal, progressively motile sperm in each of 2 ejaculates collected 1 hour apart. There are no guidelines regarding the absolute percentage of abnormal sperm or the percentage of specific defects in the ejaculate.[3]

The guidelines from the Society for Theriogenology have not taken into account the concept of uncompensable sperm traits. Sperm with uncompensable traits compete with "competent" sperm for fertilization, but are unable to sustain normal embryonic development. Therefore, pregnancy rates cannot be increased by increasing sperm numbers in the breeding dose.[75] Observations from studies in bulls suggest that disturbances in spermatogenesis extend to otherwise normal appearing sperm in the same ejaculates, but these sperm that can gain access and fertilize the oocyte are the most likely candidates for uncompensable traits causing early embryonic death.[57,58,62,72] In fact, personal experience indicates that, for example, very different pregnancy rates would be expected after using breeding doses with 15% or 65% morphologically normal sperm even if both contained the same number of morphologically normal, progressive motile sperm. It is also important to realize that breeding doses with different sperm defects might also have different effects on

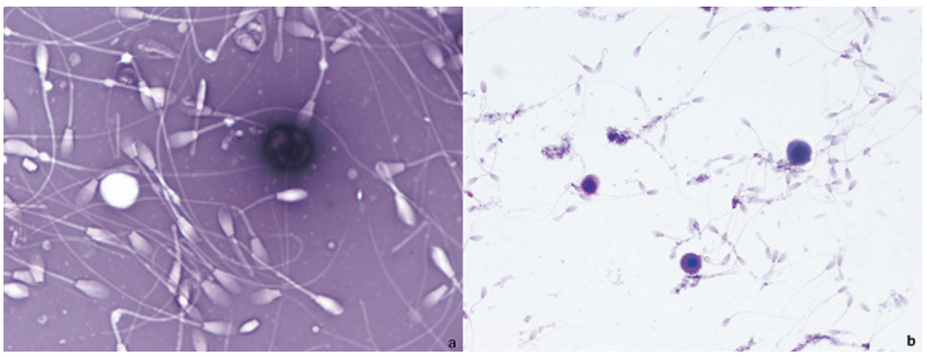

Figure 8 Membrane intact (white) and disrupted (stained) round germ cells might be observed in eosin-stained smears (a). Differentiation of these cells from WBC requires observation of round nuclei after differential staining with blood stains like the Diff-Quick (b). (Color version of figure is available online.)

fertility even when the percentages of normal sperm are the same. For example, one could expect lower fertility from a breeding dose containing 50% normal sperm, 30% sperm head defects, and 20% proximal cytoplasmic droplets when compared with a dose containing the same percentage of normal sperm but with 10% head defects, 10% DMR, 10% coiled tails, 5% detached heads, 5% proximal droplets, and 10% distal droplets. More research on pathogenesis and effects on fertility of specific sperm defects in stallions is clearly needed.

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Clinical Techniques in
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The Enlarged Scrotum

Peter R. Morresey, BVSc, MACVSc, DipACT, DipACVIM (Large Animal)

Assessment and management of an enlarged scrotum in the stallion presents a diagnostic and therapeutic challenge. Depending on the etiology and severity of the enlargement, irreversible injury may result to the reproductive system and adjacent structures. Although appearing clinically similar, scrotal enlargements vary widely in their cause. Interventions should be aimed at evaluation of the structures involved, with assessment of the degree of tissue compromise followed by timely and rational medical and surgical therapies.
Clin Tech Equine Pract 6:265-270

KEYWORDS scrotum, inguinal, testicle, congenital, orchitis, neoplasia

Scrotal enlargement of the stallion presents a diagnostic and prognostic challenge to the clinician. Although often the result of direct trauma to the scrotum, regional herniation and testicular torsion are relatively common. Less common causes include infectious agents, fluid swellings of the vaginal cavity, and neoplasia. Due to the testicle's sensitivity to vascular and temperature changes, return to fertility following scrotal enlargement is uncertain regardless of whether or not the testicle is directly involved in the inciting pathology. Therefore, scrotal enlargement of the breeding stallion requires a timely, accurate diagnosis followed by prompt therapy.

Anatomy of the Scrotum and Contents

The reproductive anatomy and physiology of the breeding stallion has been reviewed.[1] Scrotal content includes the testicles, epididymides, spermatic cords, and cremaster muscles. The scrotum is divided into two halves by the median septum, visible externally as the median raphe. Scrotal wall is composed of four layers, namely skin, tunica dartos, scrotal fascia, and parietal vaginal tunic. Scrotal skin contains a high number of sebaceous and sweat glands. The tunica dartos contains smooth muscle and fibroelastic tissue. Scrotal fascia is loose connective tissue that allows considerable movement of the inner most layer, the parietal vaginal tunic. This tunic surrounds the testicle, epididymis, spermatic cord, and is continuous with the parietal peritoneum. The visceral vaginal tunic is fused to the tunica albuginea, which encompasses the testicular parenchyma. Between the two layers of the vaginal tunic is the vaginal cavity, a small space filled with serous fluid facilitating free movement of the testicle and associated structures within the scrotum.

Testicular size varies naturally according to age, season, breed, and other factors. An exercise-oriented environment has also been shown to affect testicular size. Tone and contour are also affected by season, degree of arousal, and pathological parenchymal changes.[1]

Testicular orientation can be easily determined by palpation of the ligament of the tail of the epididymis at the caudal pole of the testicle. Rotation of 180° may be noted transiently or permanently in some stallions, and this is not associated with pathology.

Clinical Presentation of Scrotal Enlargement

During examination of the enlarged equine scrotum, the following questions should be answered to allow construction of a comprehensive differential diagnosis list leading to formulation of an appropriate diagnostic plan:

- Is the lesion congenital or acquired?
- Is the rate of onset acute and rapid, or chronic and progressive?
- Is enlargement unilateral or bilateral?
- Is there inflammation (heat, pain) associated with this enlargement?
- Is fertility normal, or is semen quality affected?
- Are there concurrent medical conditions present?

Differential Diagnoses

Scrotal enlargements may be differentiated with respect to their time of onset (acquired, congenital), degree of involvement of the scrotum and vaginal cavity, content of the vaginal cavity, and presence of any underlying testicular pathology (Table 1).

Rood and Riddle Equine Hospital, Lexington, KY.
Address reprint requests to Peter R. Morresey, Rood and Riddle Equine Hospital, PO Box 12070, Lexington, KY 40580. E-mail: pmorresey@roodandriddle.com

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Table 1 Differential Diagnosis of Scrotal Enlargement in the Stallion

Acquired	Intrascrotal	Intravaginal	Hydrocele, hematocele, inguinal or scrotal hernia (adult)
		Extravaginal	Ruptured inguinal hernia, inguinal rupture
	Scrotal wall		Neoplasia, trauma, dermatological
	Testicle	Trauma	Hematoma, fibrosis
		Torsion	
		Sepsis	Abscess, orchitis
		Neoplasia	Sertoli cell, teratoma
		Hypertrophy	
		Non-pathological enlargement	Pseudocyst
Congenital	Intrascrotal		Inguinal hernia (foal)
	Developmental anomaly		Varicocele

Time of Onset—Acquired

Acquired scrotal enlargements are not present at birth but are the result of subsequent events or disease processes. They may be further categorized with consideration of the role of the vaginal cavity, scrotum, and testicle.

Intrascrotal, Inside Vaginal Cavity

The vaginal and peritoneal cavities communicate. Spread of systemic disease or extension of disease from the peritoneal cavity may result in scrotal enlargement. Septic or nonseptic peritonitis, hemoperitoneum, and ascites can all have scrotal enlargement as part of their presenting signs (Fig. 1). In these cases, fluid is within the vaginal cavity surrounding the testicle. Management of the primary condition will lead to resolution in most cases; however, septic processes may result in adhesions and abscessation within the scrotum.

Hydrocele

A hydrocele develops when there is an abnormal accumulation of fluid between the parietal and visceral vaginal tunic. A hydrocele is not in itself pathologic unless it becomes infected. Also, transudate accumulating in the vaginal cavity may lead to pressure-induced atrophy of the testicles in some cases.

Hydrocele occurs as a primary idiopathic condition or may be secondary to parasitic migration (*Strongylus edentatus*), trauma, neoplasia, orchitis, inguinal hernia, or high ambient temperature. Hydrocele is occasionally diagnosed as a cause of scrotal swelling in stallions and is likely the result of trauma or heat stress.[2] It may also develop some time following castration, reportedly more commonly with an open technique or where insufficient vaginal tunic is separated from the surrounding fascia.[2-4]

Palpation of the scrotum reveals fluctuant fluid and small- to normal-sized testicles. Ultrasound may also be used to visualize fluid in the vaginal cavity, which typically is anechoic. If gut is visualized, the structure is more correctly called an inguinal hernia. Centesis of the anechoic vaginal cavity of a hydrocele yields serous, amber fluid. The prognosis for fertility is poor. Medical therapy includes hydrotherapy, anti-inflammatories, and diuretics; however, this is unlikely to be of long-term benefit. Castration using a closed technique of the affected testicle may be necessary if discomfort is present. Presence of remnant testicular tissue can be determined by hCG stimulation testing.[5]

Hematocele

Hematocele may present similarly to hydrocele. Ultrasonography may detect anechoic fluid, possibly with signs of fibrin content. Centesis of the vaginal cavity produces a hemorrhagic fluid. Changes in testicular weight and sperm production have been reported, likely the result of increased scrotal temperature associated with the hematocele.[6] Trauma is the usual etiology, with pain associated with palpation. Rupture of blood vessels within the scrotum or vaginal tunics leads to accumulation of blood. Adhesions may form between visceral and parietal vaginal tunics. Testicular degeneration may occur following organization of any hematoma and resultant temperature increases within the scrotum.

Inguinal Hernia

An inguinal hernia forms when intestine, usually a loop of distal jejunum or ileum with associated mesentery, passes through the vaginal ring into the inguinal canal. Depending on the degree of involvement of the inguinal canal and whether protrusion into the scrotum occurs, this condition may be designated as an inguinal or scrotal hernia (Fig. 2). With both these conditions, hernial contents remain within the parietal vaginal tunic and the scrotal fascia. Inguinal hernias of foals occur congenitally and are considered to have a heritable component. Most common on the left side, they are also reported to occur most frequently in Standardbreds. In-

Figure 1 Systemic disease (such as in this horse suffering from colitis) can result in generalized scrotal enlargement. (Color version of figure is available online.)

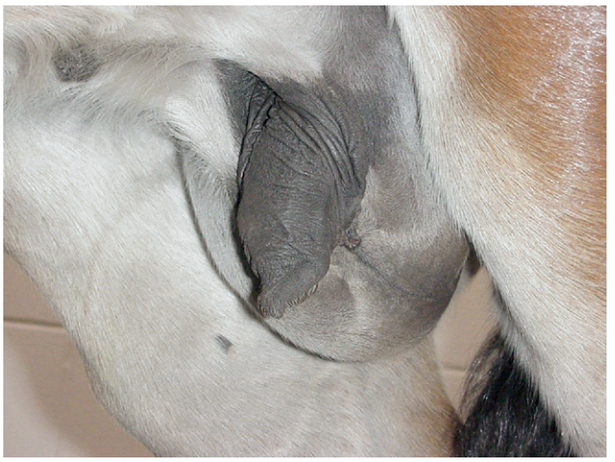

Figure 2 Passage of intestine to lie within the scrotum results in a scrotal hernia. In this case, the right hemi-scrotum is affected. (Color version of figure is available online.)

guinal hernias in adults usually occur in association with exercise and breeding activity. In contrast to congenital hernias in foals, acquired hernias in adults are usually painful, result in intestinal compromise, and are therefore surgical emergencies. The importance of early diagnosis in decreasing the mortality of affected stallions has been demonstrated.[7] Obstruction of the testicular vascular supply due to compression by the herniated structures also occurs, leading to testicular compromise (Fig. 3).

Intrascrotal, Outside Vaginal Cavity

Contained within the scrotal skin, these hernias occur when the parietal vaginal tunic, scrotal fascia, or adjacent structures rupture allowing herniated gut to leave peritoneal confinement. Small intestinal incarceration and strangulation may lead to signs of obstruction and require surgical intervention.

Ruptured Inguinal Hernia

A ruptured inguinal hernia results from similar processes to an inguinal hernia mentioned above; however; it is complicated by the passage of the hernial contents through a rent in the parietal vaginal tunic and scrotal fascia to lie subcutaneously outside the vaginal sac within the scrotal or inguinal region.

Inguinal Rupture

An inguinal rupture results from a peritoneal and transverse fascial rent adjacent to the vaginal ring, allowing viscera to pass through and lie subcutaneously outside the vaginal sac in a position similar to the above-mentioned ruptured inguinal hernia. As the intestine is not covered with peritoneum, the hernial contents may adhere to the subcutaneous tissue and also become strangulated by the fascial rent. Usually traumatic in origin, they are less common in adults than ruptured inguinal hernias.

Scrotal

Pathology of the Scrotal Wall

Parasitic invasion (Habronemiasis), cutaneous neoplasia (lymphoma,[8] squamous cell carcinoma, fibroma, sarcoid, melanoma), and dermatological infection can all lead to mass lesions or thickening of the scrotal wall. Treatment should be directed against the primary cause, as well as ameliorating as much of the associated inflammation as possible. Similarly, inflammation and infection from direct trauma can also alter the scrotal wall. Alteration of thermoregulation may lead to depressed spermatogenesis.

Testicular Pathology

Unless of an infectious nature, pathology of the testicle usually results in the necessity for removal of the affected gonad.

Trauma

Trauma is a common cause of observed scrotal swelling in the stallion. Damage may be minor or progress to scrotal contusion and testicular hematoma. Physical examination findings often include heat, pain, and swelling of the testes along with scrotal and preputial edema. A kick by the mare before, during, or after breeding or during teasing is the most common cause of orchitis in the stallion. The resulting orchitis may be unilateral or bilateral and generally remains sterile as long as no penetrating wound has occurred. Acute orchitis or hematomas may progress to become chronic, resulting in testicular degeneration and fibrosis. Compromise of the blood–testis barrier may lead to autoimmune orchitis. Following testicular trauma, sperm production capacity is rarely totally restored. If chronic inflammation can be controlled, fertility may be acceptable, albeit at a reduced level.

Testicular Torsion

Testicular torsion with subsequent thrombosis of the testicular artery is a documented cause of testicular ischemia in stallions. This ischemic insult may lead to testicular and scrotal swelling. Acute torsion of the spermatic cord may appear similar to orchitis, with colic, fever, scrotal swelling, and hind limb lameness. In contrast, chronic torsion may not have obvious clinical signs. Diagnosis is usually made with palpation of the testes. Torsion of 180° is most commonly found, with the tail of the epididymis on the cranial aspect of the testis. This is not necessarily a sign of pathology, as many stallions have this degree of rotation without problem, and it is most often only an incidental finding. Diagnosis of the less

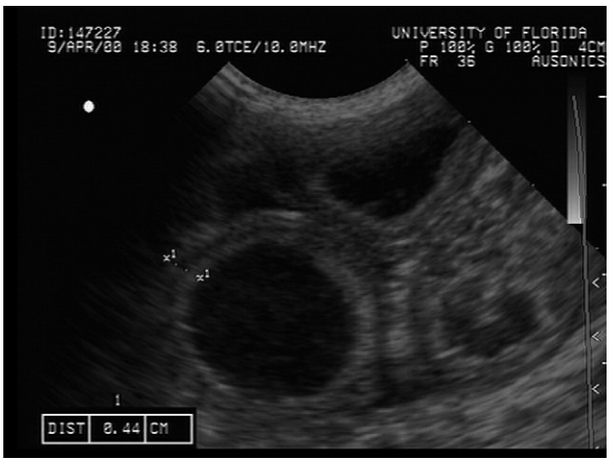

Figure 3 Same foal as Figure 2. Incarceration of the intestine within the scrotum can result is disruption of blood flow and devitalization of the intestine.

common 360° torsion is more difficult to make; however, they are surgical emergencies requiring removal of the affected testicle.[9] Manual correction of acute testicular torsion usually relieves clinical signs, but displacement tends to recur. Castration, or occasionally surgical fixation, is curative. Prognosis for full return to fertility following the ischemic insult is guarded.

Sepsis

Clinical signs of septic periorchitis include abdominal pain and an enlarged scrotum. Periorchitis has been reported to be similar in presentation to an inguinal or scrotal hernia.[10] Treatment with broad spectrum antimicrobials and anti-inflammatories allowed complete resolution of clinical signs. Abscessation secondary to other testicular pathology or adjacent lesions has been reported.[11]

Neoplasia

Neoplasia of the testes and scrotum are rare in the stallion; however, they are considered to be underreported.[12] Squamous cell carcinoma, papilloma, melanoma, and sarcoid may occur on the scrotum, although the penis and prepuce are much more common sites. These tumors might possibly cause some degree of scrotal edema and swelling.

Neoplasia involving the testicle usually manifests as an insidious enlargement unaccompanied by pain, in contrast to other causes of an infectious or inflammatory nature. The surface of the affected testicle may become irregular, and consistency may change.[9] Testicular neoplasia is rare in horses, with primary neoplasms being more common than secondary neoplasms. Tumors arise from either the germ cell layer of the seminiferous tubules or the nongerminal (stromal) testicular tissue.

Germinal cell tumors are the most commonly reported. Seminomas are the most common testicular tumors of the stallion. They are usually benign, although metastasis has been reported. In one report, ultrasonographic examination of the affected testicle revealed a diffusely hypoechoic testicle with regions of hyperechogenicity. Normal testicular tissue was not seen.[13] Of the stem cell tumors, teratomas are most common. Teratomas, the second most common testicular tumor, are benign and usually occur in cryptorchid testes; however, scrotal testicular tumors have been reported.[9] Malignant stem cell tumors include the teratocarcinoma and embryonic carcinoma. They are rarely reported.

Nongerminal cell tumors, Sertoli-cell, and Leydig-cell tumors, are uncommonly reported in the stallion.[14]

If the tumor is unilateral and has not metastasized, prognosis for return to acceptable fertility following removal of the affected gonad is good. Palpation of the associated sublumbar, inguinal, and pelvic lymph nodes is recommended before surgery to assess potential for tumor spread from the site of origin.

Endocrine (Compensatory Hypertrophy)

Following unilateral testicular degeneration or hemicastration, enlargement of the remaining testicle has been reported. This is due to loss of inhibitory feedback from the affected testicle, allowing increased stimulation of the remaining gonadal tissue.

Nonpathological Enlargements

In addition to infectious or neoplastic causes, testicular enlargement may result from relatively benign structures. Pseudocysts (fluid-filled cavities devoid of epithelial lining) have been reported.[15] Thought to be the result of sterile necrosis post trauma, they can become infected by the hematogenous route. Developmental anomalies also occur, such as cystic rete testis.[16]

Extension of Systemic Disease

Scrotal enlargement has been reported in conjunction with systemic disease. In one reported case of generalized granulomatous disease, weight loss, recurrent fever, and multifocal skin lesions were present in addition to scrotal enlargement.[17] Due to communication with the peritoneal cavity, ascites may extend into the scrotum. Generalized edematous conditions (eg, hypoproteinemia) should also be considered.

Time of Onset—Congenital

Inguinal Herniation in Foals

Inguinal hernias in foals are congenital and considered to have a heritable component. They occur when the inguinal ring is of sufficient size to allow viscera to enter the vaginal sac. Trauma at delivery with visceral herniation is the most likely cause. Inguinal hernias in foals rarely strangulate and are easily reducible due to the relatively large size of the inguinal rings. Rupture should be considered when reduction is not easily achieved, crepitous is palpable, colic is associated, or scrotal contents become cold (Fig. 4).

Varicocele

A varicocele is a congenital dilation of the veins of the pampiniform plexus. No pain is associated with this malformation. Due to disturbance of normal vascular flow, possibly due to valvular incompetence of the testicular vein, testicular swelling, and scrotal edema may develop. Examination findings include palpably thickened, irregular spermatic cords and tortuous vascular structures with ultrasound. Varicoceles in stallions are uncommon, with prognosis for normal fertility undocumented. Semen quality is likely affected by interference with testicular temperature regulation. Castra-

Figure 4 Ruptured inguinal hernia in a foal. Note the extension of the hernial content along the medial aspect of the right thigh (top of picture). (Color version of figure is available online.)

tion is the only therapy but is usually unnecessary if semen quality is appropriate.

Diagnostic Methods

History and Physical Examination

Information relating to the time and rate of onset and any activities concurrent with the occurrence of scrotal enlargement are part of a complete history. Previous or current systemic illness, fertility level, and surgical history should also be considered. Acute increase in scrotal size may result from testicular torsion, herniation, or inflammation. More chronic or progressive enlargements suggest neoplasia.

Scrotal Palpation

Direct palpation of the scrotum and its contents yields valuable information and often is sufficient to establish a working diagnosis. Scrotal wall thickness should be noted. Testicular size, shape, consistency, and temperature should be assessed. Pain in response to deep palpation should be considered an indication of pathology. Testicular and epididymidal orientation should be determined. The epididymides should lie on the dorsolateral aspect of the testicle, with the normally oriented testicle having the tail of the epididymis clearly palpable at the caudal aspect of the testicle.

Ultrasonography: B Mode, Color Doppler

Ultrasound may be helpful to determine whether the lesion is intra- or extratesticular, solid or cystic, and to evaluate lesion density relative to the surrounding parenchyma. When associated with trauma, ultrasound examination may confirm edematous thickening of the scrotum and testicular swelling. If a testicular hematoma is present in the acute stage, the lesion may be anechoic or hypoechoic due to the accumulation of blood in the testicular parenchyma. A more chronic lesion may become fibrotic and appear hyperechoic. Studies involving color doppler ultrasonography suggest possible effects of known pathology on testicular blood flow and testicular function.[18]

Palpation Per Rectum

Palpation per rectum of the internal inguinal rings may detect testicular retention, visceral herniation and incarceration, and testicular neoplasia. This procedure is better tolerated in older stallions; rectal palpation of fractious stallions is risky, and information gained may not outweigh the risk of the procedure. The presence of distended loops of intestine coursing toward the inguinal ring is highly suggestive of herniation, as is a pain response to traction. It has been suggested that palpation of the scrotum and inguinal rings should be performed on every stallion with colic.[7]

Semen Evaluation

Evaluation of the ejaculate will be of use in those cases where inflammation of the testicle is suspected, or where chronic conditions have led to significant testicular degeneration. Cytology and motility of sperm, along with assessment for the presence of abnormal cells, should be performed. Semen evaluation is noninvasive, less expensive than surgical biopsy, and gives serial information after therapeutic interventions.[19]

Aspiration

Aspiration cytology may be helpful to rule out orchitis, but usually is insufficient to diagnose neoplasia. A needle is repeatedly introduced to the testicular parenchyma at varying angles before withdrawal. Hemorrhage at the biopsy site may occur but is easily controlled by direct pressure. Aspiration cytology has been described in the investigation of fertility and allows broad cytological diagnosis.[20]

Biopsy

Biopsy of the testis may be necessary for definitive diagnosis; however, this procedure is not without significant risk. Potential complications include fibrosis, adhesions, infection, hematoma, autoimmune orchitis, and seeding of neoplastic cells to adjacent tissues. Transient alterations in seminal components and inflammatory changes at the biopsy site have been demonstrated.[21] A wedge biopsy gains the most representative sample but is the most invasive, with general anesthesia being required. Punch biopsy technique has been described.[9] Needle biopsy technique has been reviewed.[22] Parenchymal tissue is removed with minimal invasiveness, preserving normal tissue architecture. Care should be taken to avoid large vessels by sampling the craniolateral portion of the testicle.[23]

Summary

Treatment plan is dependent on the diagnosis, whether the enlargement is congenital or acquired, and if the testicle, vaginal cavity, and scrotum are involved. Congenital enlargements indicate abnormal scrotal content or disorders of gonadal structure. Acquired enlargements may result from anatomical disruptions when acute, or testicular pathology when chronic in development. Vascular compromise of the testicle and associated structures dictates the necessity for removal. Visceral herniation through the inguinal ring indicates a requirement for closure of the affected inguinal ring and removal of the ipsilateral gonad. The importance of thorough clinical evaluation, palpation of the genitalia, and relevant imaging studies cannot be overemphasized in the pursuit of a differential diagnosis of scrotal enlargement.

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Clinical Techniques in
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Evaluation of Testicular Vasculature in Stallions

Malgorzata A. Pozor

Vascular disorders play an important role in male infertility. Various modalities of ultrasound examination can be applied to evaluate testicular vasculature and to objectively measure numerous parameters of testicular perfusion. This paper describes techniques of ultrasound examination of testicular vasculature in the stallion, using gray-scale, color, and power Doppler ultrasound. Results of studies on the normal vascular anatomy in the stallion and its variations, the methods of obtaining most optimal measures of testicular perfusion, and the effects of the physiological and pharmacological factors on testicular blood flow are reviewed. There is a growing body of evidence that evaluation of testicular vasculature has a tremendous clinical relevance and should be included in the diagnostic workup of scrotal diseases.
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KEYWORDS testis, ultrasound, Doppler, artery, vasculature, varicocele

Adequate blood supply to the testis is crucial for its function. Any vascular insult significantly affects spermatogenesis, testicular volume, and may permanently impact sperm production of both the affected and contralateral testis.[1-6] In human medicine, vascular disturbances of testes are often being diagnosed and successfully treated. Varicocele is the most frequently found pathology associated with infertility in men.[7,8] This condition is usually identified in adolescent boys and treated with surgical and/or medical methods.[5,6,9,10] Torsion of spermatic cord and various scrotal injuries are emergency situations that require immediate medical attention. However, with correct diagnosis and immediate intervention, both testes are often salvaged.[11-15] Recently, various medical treatments which protect testes from detrimental effects of ischemia–reperfusion injury were described.[16-21] Introduction of these methods may further increase success of surgical procedures in traumatized testes.

We have shown that various vascular disturbances of the testes occur in stallions.[22] Unfortunately, to date, treatment of these conditions in stallions has not been investigated. Torsion of the spermatic cord and scrotal injuries do occur in stallions and most often are treated by unilateral castration to prevent permanent insult to the contralateral testis.[23-25]

The long-term goal of our current research is to find methods to improve testicular blood flow in stallions to increase testicular volume and sperm production, as well as to protect testicular tissue from a permanent damage in cases of testicular insult, such as trauma or torsion of spermatic cord.

The Role of an Adequate Blood Supply to the Testis

Good testicular perfusion is necessary for its function. Heritable testicular hypoplasia in Nguni (Bos indicus) bulls is associated with decreased blood flow and small diameter of testicular artery.[26] Experimental studies confirmed this relation. Bulls with experimentally restricted blood flow in the testicular artery had smaller testes than normal, and spermatogenesis was severely impaired.[27] Induced testicular ischemia of various degrees in rams created focal morphological changes in testes, similar to those seen among infertile men.[28] Various pathological conditions that affect testicular vasculature, such as varicocele or torsion of spermatic cord, significantly impair blood supply and testicular function. Varicocele in adolescent boys is usually unilateral, affects the left testis, and has a significant impact on the testicular volume.[5,6,29] Successful surgical repair of varicocele reverses this effect and allows so-called "cutch up" growth of the testis (small testis initially affected by varicocele increases to a normal size after varicocele repair).[6,30] Severe torsion of spermatic cord causes acute testicular ischemia on the affected side as well as impaired circulation on the contralateral side.[1-4]

Evaluation of Testicular Vasculature in Men and Stallions

Recently introduced techniques allow us to reliably evaluate the vasculature of stallion's testes and objectively measure

Department of Large Animal Science, College of Veterinary Medicine, University of Florida, Gainesville, FL.
Address reprint requests to: Malgorzata Pozor, University of Florida, College of Veterinary Medicine, P.O. Box 100136, Gainesville, FL 32610-0136. E-mail: pozorm@vetmed.ufl.edu

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testicular blood flow. Color Doppler ultrasound (CDU) is the method of choice for this application. CDU has been successfully used in human andrology for diagnosing scrotal disorders such as varicocele, testicular tumors, epididymitis, orchitis, testicular torsion, and infarction of the testis.[31-38] Specifically, CDU is helpful in assessing the distribution of blood vessels in the evaluated organ and allows direct measure of blood flow velocities within specific vessels in the various stages of the cardiac cycle (systolic velocities and diastolic velocities).[39,40] Direct measures of blood flow velocity in various stages of the cardiac cycle can be derived from waveforms representing graphic illustration of blood flow through the chosen vessel. Precise measurement of the velocity of blood flow in systole and diastole (PSV, peak systolic velocity; EDV, end diastolic velocity) may be obtained using Doppler ultrasonography. In addition, blood flow velocity can be used to calculate indices regarding plasticity and resistance of vessels and surrounding tissues. Specifically, resistive index (RI = [PSV − EDV]/PSV) and pulsatility index (PI = [maximum velocity − minimum velocity]/mean velocity) are routinely calculated during pulsed-wave Doppler evaluation of the blood flow of the organ.[39,40] In contrast to PSV and EDV, the calculated indices (RI and PI) are independent of age, bodyweight, pulse rate, and testicular volume, and therefore, significant changes in their values are usually associated with vascular pathologies.[41] From these two indices, RI has been found to be more sensitive in differentiating abnormal waveforms of the blood flow.[42] This parameter decreases when inflammatory changes are present and increases in testes of aging men, probably due to degenerative processes.[43,44] Atrophic testes of men with Klinefelter syndrome have highly resistive blood flow associated with the significantly increased values of RI.[45,46] Progressive degeneration of testicular parenchyma as well as spontaneous arteriosclerosis of testicular vessels have been described in men and in animals.[47,48] However, it is not clear whether highly resistive testicular blood flow in atrophic testes is primary due to vascular changes, or secondary to parenchymal atrophy with fibrotic, hyalinized seminiferous tubules.[46] Recently, it has been suggested that RI as well as PSV are significantly related to sperm production rate score (SPRS), whereas FSH concentration or testicular volume is not.[49] It has been concluded that PSV and RI are better indicators of an active spermatogenesis of human testis than plasma FSH concentration or testicular volume.[49] In addition, it has been shown that flow variables recorded from the capsular artery of human testis (equivalent of the marginal aspect of testicular artery in stallions) are most significantly correlated with spermatogenesis.[50] Recently, other parameters of testicular perfusion were introduced. After measuring the diameter of the artery supplying blood to the evaluated organ and time averaged mean velocity through this vessel (TAM), total arterial blood flow can be calculated (TABF = TAM × A; A = area of the cross-section of the blood vessel, calculated from the formula $A = \pi r^2$; units: ml/min). And finally, total arterial blood flow rate can be calculated using total volume of the organ (TABF rate = TABF/V × 100; V = total volume; units = ml/min/100 g). TABF rate of human testis has been shown to be significantly affected by varicocele in men.[51] Since this parameter is the best measure of organ perfusion, its changes may serve as early sign of testicular pathologies associated with vascular disturbances, which can affect testicular function and volume. Based on these compelling data in humans, CDU should be readily applied to testicular evaluation across species.

Technique of Evaluating Testicular Vasculature in the Stallion

A thorough, manual palpation of the scrotum and spermatic cord should always precede ultrasound evaluation. During this initial evaluation, an experienced operator can trace fragments of testicular artery running along the dorsal (epididymal) edge of the testis, just underneath the epididymis, and around the caudal pole of the testis, just underneath or lateral to the epididymal tail. Multiple convolution of this vessel can also be palpated within a spermatic cord. In some cases, additional branch of the testicular artery can be detected by palpation on the lateral surface of the testis. In mature stallions, a presence of this additional vessel is associated with a distinct bulging on the surface of the testis, due to the trophic effect.

Ultrasound evaluation of testicular vasculature in the stallion can be performed using a good quality machine, equipped with color or power Doppler ultrasound modality. To objectively measure parameters of testicular perfusion spectral mode (pulse wave mode) is also necessary. There are many portable ultrasound machines with optional Doppler and spectral modalities currently available on the market. However, only a limited number of transducers can be used for spectral modality. Linear transducer with broad range of frequencies is most useful for evaluating scrotal contents in the stallion, including vasculature. It can be easily positioned between stallion's leg and the scrotal surface, close to the testis, epididymis, or spermatic cord. Other transducers (convex, microconvex, or sector) can be used also; however, it is more difficult to manipulate such probes in the tight, inguinal area.

The majority of stallions can be examined without heavy restraint or tranquilization. Stallion can be positioned in the corner of the large examine room, with one side and the hindquarters close to the walls. Placement of heavy padding on the wall behind the stallion may help prevent leg injury due to the kick. This examination can also be done in the box stall, where the stallion is being housed. Stallion should be positioned with his left side facing the open door, while the ultrasound machine and the operator are outside the stall, protected by the stall door. If the stallion is placed in stocks, one side has to be open, and/or side bar should be elevated to allow good access to the scrotum. Caution should be always applied, and tranquilization should be used when needed. Do not use phenothiazine tranquilizers in stallions. To record the parameters of testicular perfusion additional measures may be necessary. This part of the examination is time consuming and requires patience of the operator and cooperation of the animal. Some stallions stand quietly for an adequate amount of time when simultaneously fed with grain or hay.

After thorough palpation of the scrotal contents and determination of the stallion's reaction to manipulations, a gener-

Figure 1 Gray-scale ultrasound images of testicular vasculature in the stallion. (a) Central vein (cross-section of the testis). (b) Marginal part of testicular artery (short longitudinal section on the caudal pole of the testis). (c) Convoluted part of testicular artery (cross-section of the spermatic cord).

ous amount of the lubricant is placed on the skin of the scrotum. The operator places the transducer on the lateral scrotal surface, perpendicular to the long axis of the testis. A round cross-section of the entire testis should be visualized this way. The parenchyma of the normal stallion testis has a homogenous echotexture of medium echogenicity. Central vein is easily found on this background as a hypoechoic dot (cross-section) or wavy line (longitudinal section) (Fig. 1a). This vessel starts in the center of the caudal pole of the testis and courses cranio-dorsally to enter the spermatic cord. Testicular artery and veins can be also visualized, using gray-scale ultrasonography, on the epididymal (dorsal) edge of the testis, just underneath the body of the epididymis, or on the caudal pole of the testis, underneath the tail of the epididymis (Fig. 1b). Ultrasound image of arteries is anechoic, whereas the veins have a hypoechoic sonographic appearance. Additional branches of testicular artery, running on the lateral surface of the testis, can be detected with the ultrasound and have similar diameter as the main artery. Identifying this anatomical variation is highly recommended before performing testicular biopsy, since usual site of this procedure may be on the path of the large, arterial vessel. Small arteries, branching from the main vessel and entering testicular parenchyma can also be seen on the free (ventral) edge of the testis.

The transducer should be moved slowly along the long axis of the testis, with the ultrasound beam directed medially. Round cross-sections of the entire testis are visualized this way. Transducer is slowly turned when approaching the cranial pole of the testis until it is parallel to the long axis of the testis, and slowly moved toward the external inguinal ring along the spermatic cord. Multiple cross, oblique, and longitudinal sections of a convoluted part of the testicular artery appear as anechoic dots and lines coursing in various directions (Fig. 1c). Clear pulsation of this vessel is obvious. In rare cases, the testicular artery may divide into two or three vessels on various levels of the spermatic cord. Veins of the pampiniform plexus have very small diameters and are not as easily identified as the artery. Larger veins as well as lymphatic vessels can be seen on the periphery of the spermatic cord.

After identifying all the vascular structures using gray-scale ultrasound, color Doppler mode is used. A box appears on the screen which shows the area where various velocities of blood flow within the vessels is color-coded and displayed on the screen. The direction of blood flow is assigned the color red or blue, indicating flow toward or away from the ultrasound. Different hues of these colors refer to different velocities. A bar with a scale of these colors and velocities appears on the side of the screen. The ultrasound should be set for low velocities. Distribution of small vessels within testicular parenchyma should be evaluated. They appear as multiple dots or very thin lines running through the parenchyma. Due to very low velocity of blood flow within these vessels, it may be difficult to detect them using color Doppler ultrasound. Power Doppler ultrasound mode may be helpful in visualizing them. This technique depicts the amplitude or power of Doppler signals rather than the frequency shift. This allows better visualization of small vessels, but at the expense of directional and velocity information. There is no color coding of various velocities in power Doppler mode, and blood flow appears in various undertones of orange color. Distribution of blood flow should be uniform within testicular parenchyma (Fig. 2). Focal increase in Doppler signal may be indicative of inflammatory or neoplastic process, whereas lack of the signal may suggest ischemia. The course of the testicular artery can be easily traced using color Doppler ultrasound. The convoluted portion of this artery in the spermatic

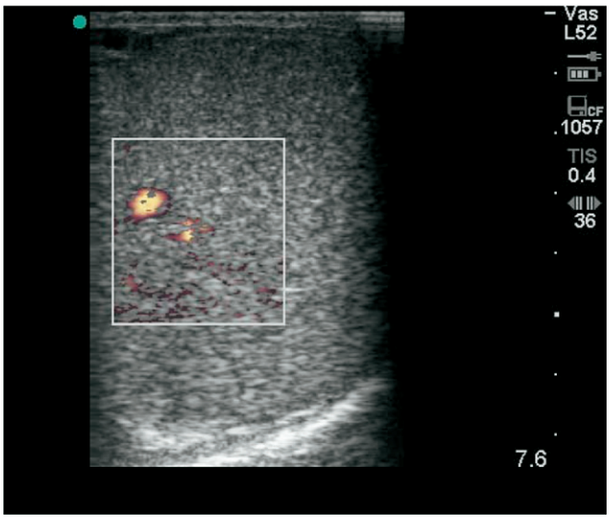

Figure 2 Power Doppler ultrasound image of central vein and small, intratesticular vessels.

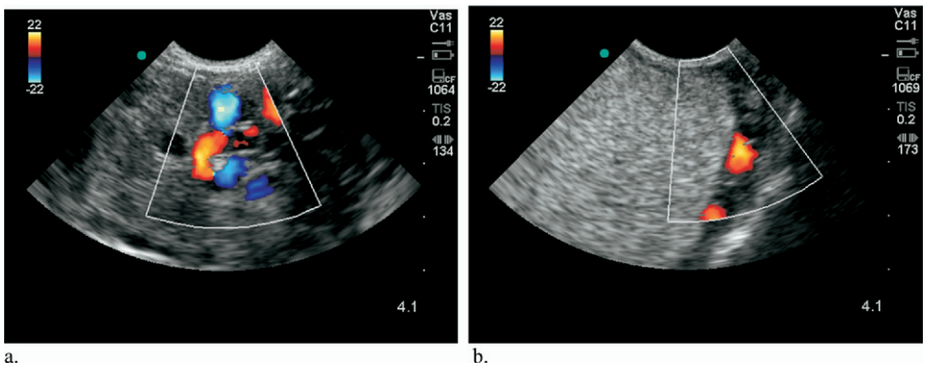

Figure 3 Color Doppler ultrasound images of testicular artery. (a) Convoluted part (spermatic cord). (b) Marginal part (caudal pole of the testis).

cord appears as an "entanglement" of thick red and blue dots and lines, crossing over, making multiple turns, and intensely pulsating (Fig. 3a). The marginal part of this vessel can be identified on the epididymal edge of the testis and on the caudal pole of the testis. Its course in these areas is not very tortuous, which allows visualization of longitudinal sections of this vessel, which is necessary to obtain representative measures of testicular perfusion (Fig. 3b). We have described the technique of obtaining various measures on three different levels of testicular artery in the stallion (spermatic cord, proximal and distal fragment of marginal part).[52] However, our next studies showed that the most reliable measures can be obtained from the marginal part of testicular artery, on the caudal pole of the testis at the level of epididymal tail.[53,54] Linear or microconvex transducer can be used; however, microconvex transducer provides wider variety of angles of the ultrasound beam, which helps in obtaining optimal insonation for spectral analysis.

For spectral analysis of the blood flow, a small gate size (1-2 mm) and angle correction between 30° and 60° should be set. After activating pulsed-wave mode (PW), a line with a small gate will appear on the screen. This gate has to be placed within the lumen of the longitudinal section of the vessel, running parallel to the line. The best insonation is reached when a direction of the blood flow is parallel to the ultrasound beam. The operator can manipulate with the position of the probe to improve insonation. In addition to it, angle correction mode can be used. Pressing PW button again activates spectral analysis of blood flow velocities. A characteristic wave form of testicular blood flow in the stallion is biphasic with a high, systolic peak and a lower, diastolic peak (Fig. 4a). It may be also monophasic, with just one, systolic peak (Fig. 4b). Biphasic spectrum is characteristic for the proximal parts of the testicular artery (convoluted part – spermatic cord), and monophasic spectrum is often identified in distal parts of this vessel (marginal part). Direct measures of blood flow velocities are obtained from the graph (PSV, peak systolic velocity; EDV, end diastolic velocity; MV, minimum velocity). Additional parameters are calculated using the ultrasound, built-in algorithm (TAM, time averaged maximum velocity; RI, resistance index; PI, pulsatility index).

To calculate parameters of testicular perfusion, a diameter of testicular artery and testicular dimensions have to be measured. The length, height, and width of each testicle are measured using B-mode ultrasonography and specific orientation of the transducer. Total testicular volume is calculated using the formula for ellipsoid volume: $[V_t = (4/3\pi)(W/2)(H/2)(L/2)]$ (where W = width, H = height, and L = length).[55,56] And finally, testicular perfusion parameters are calculated: cross-sectional area of testicular artery (A): $A = \pi r^2$; (r = arterial diameter/2); Total Arterial Blood Flow (TABF): TABF = TAM × A (units = ml/min); Total Arterial Blood Flow Rate (TABF rate): TABF rate = $TABF/V_t \times 100$ (where V_t = total testicular volume) (units = ml/min/100 g).

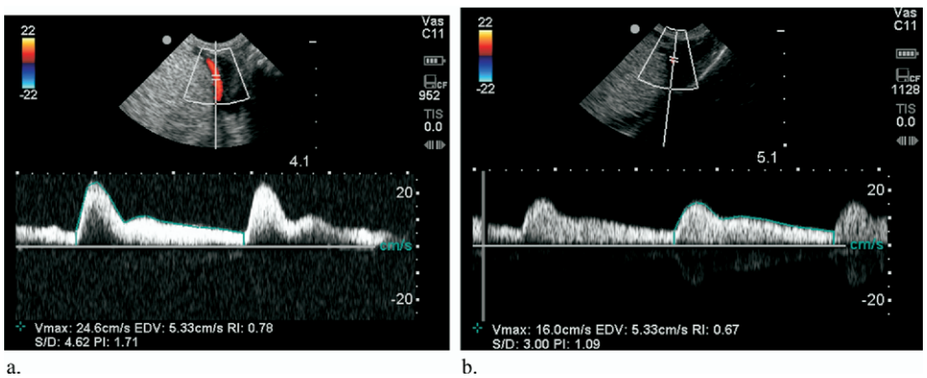

Figure 4 Spectral wave-forms of testicular blood flow in the stallion. (a) Biphasic wave-form. (b) Monophasic wave-form. (Color version of figure is available online.)

Results of Our Studies on Testicular Vasculature in Stallions

In recent years, we have performed a series of studies on testicular vasculature in stallions, which showed that there is a clinical relevance in evaluating testicular perfusion using available methods.

Morphological Evaluation of Testicular Artery in Stallions

Our initial studies were focused on anatomical aspects of arterial blood supply to stallion's testis. Two graduate students performed evaluation of 187 stallion testes obtained from the abattoir. Of these testes ($n = 157$), 84% had 1 testicular artery supplying arterial blood to the organ, whereas 14% of these testes ($n = 26$) had 2 arteries, 1 running around a caudal pole of the testis and another 1 localized on the in one-half or one-third cranial part of the lateral side of the testis.[57,58] Only 2% ($n = 4$) of these testes had 3 testicular arteries, 1 running along the epididymal edge and caudal pole and 2 on the lateral side of the testis. Interestingly, additional, lateral arteries had a strong, trophic effect of testicular parenchyma. All testes obtained from mature stallions that had a single artery ($n = 90$) had ellipsoid shape, whereas testes with double or triple arteries ($n = 11$) had pear-like shape with a visible bulge on the lateral side where additional arteries were present. This feature was not observed in testes obtained from young foals. These studies also showed that testicular artery may divide into 2 or 3 separate vessels on the various levels of the spermatic cord (before or within convoluted part).

Ultrasound Evaluation of Testicular Artery and Veins in Stallions

Gray-scale ultrasound evaluation of testicular vasculature was initially performed in 17 mature stallions.[57] Diameters of testicular artery and central vein were measured. Average diameter of testicular artery in the area of the caudal pole of the testis was 4.5 mm (2.5 to 5.3 mm). An additional testicular artery coursing on the lateral surface of the testis was detected with the ultrasound in 4 stallions and had similar diameter as the main artery (3.3, 3.3, 4.6, and 5.3 mm). The central vein was measured in the middle part of the testis. It was localized most often in the dorsal third or less frequently upper half of the testis. Its average diameter was 2.8 mm (1.8 to 5.4 mm). Ultrasound was then applied to evaluate internal and external genitalia in a large group of stallions. One hundred normal breeding stallions and 13 stallions with fertility problems were evaluated using B-mode ultrasonography.[22] Arterial and venous vessels in the spermatic cord were also evaluated. In both groups, varicocele was the most frequently diagnosed abnormality (9% in normal stallions and 15% in stallions with fertility problems). The lesion appeared ultrasonographically as irregular echolucent areas, usually on the periphery of the spermatic cord, with no signs of pulsating blood flow. The average size of the varicocele was 15.5 mm (8 to 24 mm). In 1 case, the central vein and its smaller branches were dilated while the veins within the pampiniform plexus were only slightly enlarged.

Doppler Ultrasound Evaluation of Stallion Testes

In our initial study on testicular blood flow in stallions, gray-scale Doppler ultrasonography was used.[59] Application of this technique let us measure blood flow parameters within the testicular artery in 6 stallions but was not useful for evaluation of distribution of small intratesticular vessels. Also, visualizing appropriate fragments of the testicular artery for spectral analysis was difficult. Our more recent study conducted on the population of 52 breeding stallions showed that color Doppler ultrasonography (CDU) helps to identify the best locations of elongated fragments of the testicular artery and improve the angle between a blood vessel and the ultrasound signal (insonation). This angle should not exceed 30° to 60°.[39,40] With better insonation, CDU improved the accuracy of blood flow measurements. Furthermore, CDU allowed visualization of small intratesticular vessels.[52] From these data, we have described characteristics of typical waveforms of testicular artery in the stallion. Also, we have established baseline reference values of blood flow measures and indices in the stallion testis (PSV, EDV, PI, and RI). And, in addition, we have shown that there is an effect of age and some scrotal pathologies on selected blood indices in stallions.[52]

Physiological Factors Affecting Testicular Blood Flow in Stallion

More recently, we have studied a seasonal effect on testicular blood flow in five mature, fertile stallions.[53] For this project, we have introduced parameters of testicular perfusion (total arterial blood flow, TABF; unit = ml/min; and total arterial blood flow rate, TABF rate, unit = ml/min/100 g testis). Five mature stallions were evaluated during the winter, spring, summer, and fall. B-mode ultrasonography and CDU were used to measure testicular dimensions and to perform spectral analysis of testicular blood flow in the marginal part of testicular artery. All of these evaluations were performed within the same window of time (1-3 PM) to avoid the effect of a possible diurnal fluctuation of testicular blood flow. There appeared to be a significant increase in testicular blood flow in these stallions in spring months in comparison to winter months. This effect decreased already as early as in July. This study also showed that there is a positive correlation between testosterone concentration and arterial diameter, TABF and TABF rate (Figs. 1 and 2). Estradiol concentration was significantly correlated with EDV, PI, RI, and age.

Currently, we are investigating the effect of social status of stallions on testicular blood flow. Our studies, performed during two consecutive breeding seasons, consistently showed that there is a difference between harem stallions and bachelor stallions. Harem stallions had significantly higher values of blood flow indices in comparison to bachelors (Pozor and McDonnell, unpublished data).

The Effect of a Single Administration of hCG on Testicular Perfusion in Stallions

Similarly to our previous experiment, five mature stallions were used. Each stallion received each of the five treatments

with hCG or saline (control treatment): 500 IU, 1000 IU, 2500 IU, 6000 IU of hCG, and 2.5 mL of saline. There was a 1-week interval between administrations of different treatments for each stallion. Stallions were examined just before the treatment (0 hour), and at 1 hour, 72 hours, and 7 days after treatment.[54] Both scrotal testes of each stallion were evaluated using B-mode and Color Doppler ultrasound using a portable ultrasound unit with a 4- to 7-MHz curved array transducer (Tytan; SonoSite, Inc., Bothell, WA). All parameters of testicular blood flow were obtained, and measures of testicular perfusion were calculated as described previously. Testosterone concentrations in blood serum were determined using chemoluminescent immunoassays.

There was a significant increase in testicular perfusion (TAM and TABFR) at 1 hour post administration of the highest dose of hCG (6000 IU). This effect was decreased by 72 hours. There was no effect of any of the treatments on any of the parameters at 7 days post administration of hCG. Administration of saline (control treatment) did not have any effect on the blood flow parameters or testosterone concentrations. Testosterone concentration significantly increased after each of the administrations of hCG. This effect was still present at 72 hours after administration of 2500 IU of hCG and significantly greater after administration of 6000 IU of hCG. There was a statistically significant correlation between testosterone concentration and PSV, EDV, TAM, TABF, and TABF*R* ($P < 0.001$).

These results suggest that treatment with a single administration of 6000 IU of hCG may be helpful in improving testicular perfusion in stallion testes.

Conclusions

There is a great interest in the equine industry to improve sperm production in stallions, especially in individuals with impaired fertility. Because testicular volume is directly correlated with sperm production, increasing testicular blood flow should be effective in increasing sperm production. Also, the possibility of salvaging an injured testis by surgical or medical treatment may be of great importance for equine breeders. Breeding accidents do occur, especially in Thoroughbred stallions. Trauma to the stallion's scrotum may cause significant damage of stallion's testis or testes. It is our clinical experience that testicular blood flow may be impaired by hydrocele or traumatic hematocele with rupture of the proper ligament of the testis and restored to normal in contralateral testis after removal of the affected one. In human medicine, similar or much worse conditions are treated and both testes are often salvaged.[12-16] Testicular salvage may be more protective to overall testicular function compared with unilateral orchiectomy of the traumatized testis.[15] The overall goal of our future research is to investigate similar possibilities in veterinary medicine. Protective effects of medical therapy that improves testicular blood flow may be very useful in delaying tissue damage of both the injured and contralateral testis.

Therefore, further research on application of imaging techniques, such as CDU, in diagnosing early vascular disturbances of stallion's testes should be continued. Also, future studies should be focused on the development of new, effective therapeutic strategies which could be applied to improve sperm production in stallions, salvage traumatized stallion testes, or repair vascular damage.

Acknowledgments

The images have been obtained using Sonosite Titan ultrasound system, equipped with 8- to 5-MHz curved array (microconvex transducer) (Ultrasource, Inc., Grand Rapids, MI), as well with the linear 10- to 5-MHz transducer, kindly provided by Mr.Chuck McCrary (Aloka, St.Petersburg, Fl).

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Pathogenesis, Diagnosis, and Management of Testicular Degeneration in Stallions

Regina M. Oristaglio Turner, VMD, PhD, Diplomate ACT

Testicular degeneration is a common cause of subfertility and infertility in stallions. The disease can broadly be divided into two categories: those cases resulting from a known testicular insult, and idiopathic (senile or age-related) testicular degeneration. This manuscript describes the problem of testicular degeneration in the equine breeding industry and summarizes what is known about the pathophysiology of the disease. Additionally, the clinical signs of testicular degeneration are reviewed so that the clinician can more quickly and accurately arrive at a diagnosis. Differences in the approach to treatment of testicular degeneration arising from a known cause and idiopathic testicular degeneration are discussed as are differences in prognoses. Finally, the practitioner is provided with practical information on how to more effectively manage affected stallions and what, if anything, can be done to improve reproductive performance of these animals both in the field and in a referral setting.
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Testicular degeneration (TD) can be defined as that process which causes a deterioration in the structure of the testis with a consequent loss of testicular function. In stallions, TD is a common cause of acquired subfertility and infertility.[1,2] As such, economic losses resulting from this disease in the equine breeding industry are substantial and stem from losses of breeding fees, increased management costs, and loss of valuable male genetics. However, despite its importance, the pathophysiology of the disease remains poorly understood.

In some cases, TD arises acutely secondarily to a known insult to the testis. For example, testicular trauma, exposure of the testis to heat, cold, radiation, toxins, or ischemia, certain nutritional deficiencies, administration of exogenous androgens, infection, autoimmune disease, sperm outflow obstructions, and neoplasia all can lead to TD in the horse.[3-6] In these cases, the extent of TD is often determined by the length and the severity of the causative insult. If the testis is only mildly affected, some areas may recover once the insult is removed. Even if the testis is severely affected, TD generally does not progress if and when the inciting cause is removed.

However, in other cases, stallions are affected by TD with no identifiable underlying cause. This type of degeneration, also called idiopathic TD (ITD), is most often seen in middle-aged or older stallions (senile or age-related testicular degeneration) but also can affect much younger animals.[7-9] Regardless of the age of onset, ITD is typically progressive and results in a steady decline in fertility, sometimes ending in sterility. The two different categories of TD (eg, arising from a known insult versus idiopathic) should be considered separately as the treatment and prognosis for these two groups of cases are quite different.

Pathogenesis

Testicular degeneration can be focal or diffuse and can affect one or both testes.[2] Generally, focal and/or unilateral TD is associated with some sort of traumatic insult, local infection, or neoplasia, whereas systemic insults (eg, toxins, exogenous androgens, nutritional deficiencies, etc.) and ITD more commonly uniformly affect both testes. The pathogenesis of each of these conditions varies widely. For most of the known causes of TD, the pathogenesis has previously been well described in the stallion and in other species.

Blanchard and Johnson[10] reported increased germ cell degeneration rates in stallions producing low sperm numbers. The germ cell loss is especially evident during early meiosis and spermiogenesis. Additionally, a lower germ cell:Sertoli cell ratio was reported in these stallions. These endpoints may be common to all cases of TD, even though the original inciting incident may vary. In general, earlier stage germ cells (eg, spermatogonial stem cells) and testicular somatic cells (Leydig and Sertoli cells) appear to be more resistant to de-

Department of Clinical Studies, New Bolton Center, University of Pennsylvania School of Veterinary Medicine, Kennett Square, PA.
Address reprint requests to Dr. Regina Turner, New Bolton Center, 382 West Street Rd., Kennett Square, PA 19348. E-mail: rmturner@vet.upenn.edu

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generative changes. Thus, if the inciting cause of TD can be identified and removed, these remaining germ cells may have the ability to repopulate the testis with normal spermatogenesis. Thus, for example, in cases of heat-induced TD, once the testes are returned to a physiologic temperature, normal spermatogenesis often resumes. One also can extrapolate from this information that, in those uncommon cases when a testicular insult is so severe as to cause permanent damage even to the earliest germ cell stages (such as spermatogonial stem cells), then spermatogenesis may not recover and the damage to the testis may be permanent.

There is less information available on the pathogenesis of ITD in the stallion. Several investigators have studied endocrine changes in aging, subfertile stallions,[11-14] many of which were probably affected by ITD. These reports suggest that a stallion's endocrinologic status can vary based on the nature of the individual's subfertility and the severity of the problem. In general, subfertile animals with more mild testicular changes showed no consistent, statistically significant changes in plasma hormone concentrations compared with normal, fertile animals. More severely affected subfertile animals and infertile animals had elevated plasma FSH and LH concentrations, lower plasma estradiol concentrations, and lower plasma and intratesticular inhibin concentrations. It has been suggested that low-plasma estrogens in the presence of high-plasma FSH is associated with low fertility and possibly testicular degeneration.[12] These and other studies designed to test hypothalamic, pituitary, and testicular function in fertile, subfertile, and infertile stallions all indirectly suggest that the testis, rather than the hypothalamus or pituitary, is the primary problem in cases of idiopathic stallion infertility.[15]

If a primary testicular defect is present in stallions with ITD, it appears to reside downstream of the LH receptor since it has been shown that the number of LH receptors in the testes of fertile and subfertile stallions is not different.[16] Perhaps a defect in the steroidogenic pathway itself is involved.[15] It has been reported that a decline in testicular inhibin concentrations is the first observed change in testicular steroid levels in subfertile stallions. This suggests that, in cases of idiopathic infertility, the primary defect resides in the Sertoli cell rather than in the Leydig cell.[14] Regardless of the primary cell type that is involved, these studies supply further indirect evidence to support the hypothesis that the primary cause of ITD resides at the level of the testis, rather than in the hypothalamus or pituitary.

Recently, we used the novel technique of testis tissue xenografting to directly test the hypothesis that, in cases of severe ITD, the testis itself is defective. Xenografting involves transferring small (1-2 mm^3) pieces of testicular tissue from a donor animal (in this case, stallions affected by severe ITD) under the back skin of immunocompromised, castrated mice. The mice then act as bioincubators for the tissue and, when the testis itself is normal, equine spermatogenesis is reconstituted within the xenografts.[17] In contrast, when ITD tissue is xenografted onto mice, spermatogenesis does not develop. In fact, these grafts undergo further rapid and severe degeneration.[18,19] This finding strongly suggests that, at least in severe cases of ITD, the testis itself is defective since placing the testicular tissue into the normal, permissive environment of the mouse does not correct the pathology. In addition, host mice carrying xenografts were treated with exogenous gonadotropins (PMSG and hCG) or endogenous hormones supplied by fully functional pig testis grafts cografted with the diseased equine tissue. None of these treatments changed the outcome; all equine ITD grafts degenerated rapidly.[18,19] These data suggest that equine testes that are severely affected with ITD are unable to respond to either exogenous or endogenous hormones. Taken one step further, these data suggest that treatment of stallions affected with severe ITD (in particular hormonal treatment) is unlikely to improve the condition.

Clinical Evaluation

History

A complete history is necessary before one can make an accurate diagnosis of TD since both clinically and histologically TD may be indistinguishable from testicular hypoplasia. Since TD is an acquired condition, whereas testicular hypoplasia is congenital, a firm diagnosis of TD can be made only if the stallion has a history of declining reproductive efficiency, decreasing testicular size, decreasing semen quality, or some combination of these things. As such, information on the stallion's past book sizes, seasonal pregnancy rates, average numbers of heat cycles per pregnancy, testicular measurements, and past semen analyses all can be very helpful. It should be kept in mind that many animals with testicular hypoplasia often are affected by degeneration as well.[2]

A thorough history also may reveal a cause for the TD. For example, the history may include an incident of testicular trauma, a history of recent illness associated with fever, administration of anabolic steroids, or administration of other potentially damaging substances. In cases of traumatic or thermal injury to the testes, the onset of infertility is generally sudden and closely associated with the inciting incident. If steroids or other harmful agents are involved, the progression of the problem may be acute or more gradual. In cases in which a history reveals a likely inciting cause, removal of the cause can allow for restoration of testicular function. Thus, unless the spermatogonial stem cells have been permanently damaged, the prognosis for future fertility is generally better than for cases of ITD.

If a stallion presents with a history of declining fertility over time with no apparent inciting incident, then ITD should be suspected, particularly in older animals. Although classic ITD is considered to be a slowly progressive problem, some cases may present for a perceived acute onset of infertility or subfertility, particularly if semen quality and testicular parameters were not being routinely monitored.

Clinical Signs and Diagnosis

For cases in which TD results from a known, finite cause (such as an increase in scrotal temperature secondary to fever or trauma to the scrotum), azoospermia may be seen within the first 2 weeks after the insult. One or both testes may be affected, depending on the cause. Other signs associated with the inciting incident, such as scrotal edema, hydrocele, hematoma formation, etc., also may be apparent. If the inciting factor is removed, semen quality may improve gradually over

the next 2 months. In severe cases, it may take up to 5 months for complete recovery and return to normal sperm production.[1] However, the resistant nature of spermatogonial stem cells and testicular somatic cells (Leydig cells and Sertoli cells) to injury usually provides for a population of cells that are capable of repopulating the testis. It is difficult to predict to what degree testicular function will rebound. In some instances, fertility returns to the same level as was present before the incident. In other cases, the recovery may only be partial. In very severe cases when the stem cells or somatic cells themselves are permanently damaged, there may be no recovery.

Cases of ITD generally present with a range of clinical signs. Mild cases of ITD may not be associated with any noticeable change in testicular character. Specifically, studies on germ cell loss rates in stallions indicate that ITD can be present before any clinically significant decrease in testicular size can be appreciated.[10] As such, early signs of ITD may only be noticed if semen quality is being frequently and carefully monitored. A gradual decline in overall semen quality (including a decline in total sperm numbers and/or declines in the percentages of motile and morphologically normal sperm) may be the only clinical signs early in the disease. As the disease progresses, clinical signs become more apparent and include decreasing testicular size [most often affecting both testes similarly (Fig. 1), but infrequently affecting one more than the other], palpable softening of the testicular parenchyma, decreasing sperm numbers, low Daily Sperm Output (DSO) per milliliter of testis, the appearance of increasing numbers of immature round spermatogenic cells, and/or multinucleate giant cells in the ejaculate and an overall decline in semen quality.[2,3,7,20] In advanced cases, stallions may become azoospermic. Because the size of the epididymis usually does not change in cases of ITD, the epididymis may seem to be disproportionately large with respect to testicular size.[1] It is the author's experience that, if a stallion's fertility is not regularly monitored, some cases of ITD present for what is perceived to be an acute onset of subfertility or infertility. In fact, in many of these cases, the problem was more likely progressive over time but went unnoticed until it had become a severe problem. In severe, end-stage ITD, the testicles may become overly firm.[21]

Primary Care Management and Diagnostic Techniques

In stallions that can be followed over time, it is recommended that testicular measures be obtained at least annually. At some top farms, the testes of valuable stallions are measured monthly. Measurements can be obtained either with calipers or ultrasonographically and should include measurement of total scrotal width, and length, width, and height of each testis. Length, width, and height measures then should be used to calculate testicular volume and to determine whether the stallion is producing appropriate sperm numbers for his testicular size. The volume of a single testis can be calculated using the following formula:

$$4/3\ \pi \times \left[\text{length of testis (cm)}/2 \times \text{width of testis (cm)}/2 \times \text{height of testis (cm)}\right]/2$$

And total testicular volume equals:
volume of the left testis + volume of the right testis.[22]

Additionally, DSO per milliliter of testis can be calculated by dividing the total number of sperm in the ejaculate at DSO by the total testicular volume. Low DSO/mL of testis together with a low percentage of morphologically normal sperm in the ejaculate have been recommended as good indicators of the possible presence of TD.[22]

Frequent examination and measurement of the testes facilitate early identification of trends suggestive of ITD (eg, decreasing testicular size/volume). Semen analysis also should be performed at least annually and, when possible, much more frequently (ie, for stallions breeding by artificial insemination, each ejaculate should be analyzed). However, this may not be practical for Thoroughbred stallions breeding exclusively by natural cover.

Management and Diagnostic Techniques at Referral Institutions

Stallions at sexual rest typically have large epididymal stores of sperm. Therefore, until this sperm reserve is essentially exhausted or stabilized, total sperm numbers at sexual rest can be highly variable. Therefore, gradual downward trends in total sperm numbers and semen quality will be more difficult to identify if ejaculates are only examined when the stallion is at sexual rest. A better option is to examine the

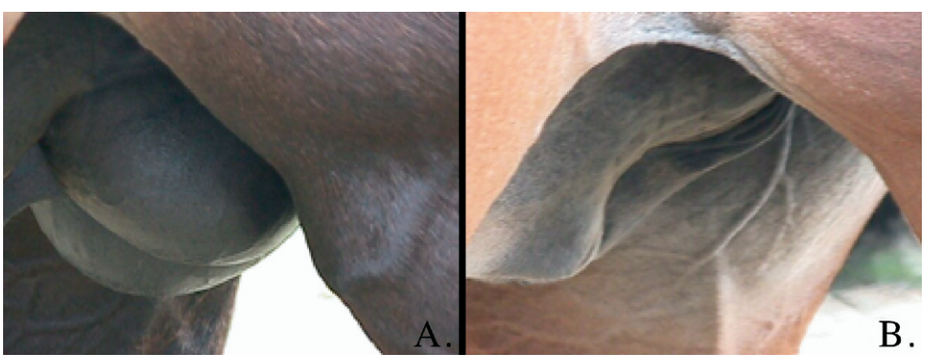

Figure 1 Clinical presentation of a stallion with normal testes and one affected with severe, bilateral idiopathic testicular degeneration. (A) Normal testes. The testes are symmetrical and of good size. (B) Severe idiopathic testicular degeneration. Note that both testes are affected and are dramatically decreased in size. (Color version of figure is available online.)

stallion's semen quality after sperm reserves are depleted (ie, when the stallion has reached DSO). This requires serial semen collections (2-3 ejaculations per day for 3-5 days). Once DSO is reached, ejaculated sperm numbers become more consistent, thus making subtle changes in sperm numbers and semen quality more apparent. ITD might be suspected if a stallion's total sperm numbers or semen quality is declining progressively over time or if a stallion at DSO is producing low sperm numbers for his testicular volume.

Another hallmark of TD (whether it results from a known cause or is idiopathic) is the appearance of immature spermatogenic cells (round cells) in the ejaculate. In an unstained semen sample, these cells can sometimes be confused with white blood cells. However, because different stages of spermatogenic cells typically appear in a single ejaculate, spermatogenic cells usually vary in size, whereas white blood cells are more homogeneous. Analysis of a Diff-Quik-stained semen sample can facilitate identification of neutrophils and lymphocytes and so, by process of elimination, can aid in the identification of spermatogenic cells (Fig. 2). Multinucleated giant cells also may be present.[2,3] Keep in mind that low numbers of immature spermatogenic cells may be found in the ejaculates of normal stallions.[23] However, in normal stallions, other signs of abnormal spermatogenesis (small testicular size, poor semen quality, low sperm numbers, etc.) should not be found.

Because of the variation in plasma hormone levels seen in normal and subfertile stallions, circulating hormone levels may not be a good predictor of mild to moderate TD.[10] In severe cases, elevated FSH and LH as well as low plasma estradiol are consistent with a diagnosis of TD.

Ultrasonographic evaluation of testes affected by ITD is often unrewarding since the ultrasonographic appearance usually is not remarkable. Nonetheless, ultrasonographic evaluation of the testes is recommended both to obtain accurate testicular measurements and to rule out possible inciting causes of TD or other testicular pathologies.

Histopathology of affected testes reveals a common group of spermatogenic abnormalities, including cytoplasmic vacuolization and a loss of the normal architecture of the seminiferous epithelium.[3] The diameter of the seminiferous tubules may be decreased, and immature spermatogenic cells may be shed into the lumen of the seminiferous tubule (Fig. 3). In more severe cases, these immature (or "round") spermatogenic cells may appear in the ejaculate in increasing numbers, as described above (Fig. 2). As TD progresses, there is an increased loss of germ cells from the seminiferous tubule. In the most extreme cases, fibrous tissue may be present and tubules can become almost devoid of spermatogenic cells and may be left with only Sertoli cells and few spermatogonia (Fig. 3). Fibrosis and calcification of the testicular parenchyma also may be seen.[24] Keep in mind that even normal testes can have some focal areas of abnormal spermatogenesis. Thus, the percentage of the testicular parenchyma that is affected as well as the severity of the histological lesions should be taken into account before a diagnosis of TD is made.

Because histopathologic findings can help to define TD (and testicular hypoplasia), evaluation of a testicular biopsy sample does provide definitive evidence of these conditions. However, in practice, testicular biopsy is rarely indicated. Once the clinician has obtained an adequate history and has performed a complete physical and reproductive examination, a diagnosis of TD can usually be made with some confidence and a biopsy sample is not necessary. Additionally, there is some concern that a single biopsy sample may not be representative of the condition of the entire testis and thus may not be of significant prognostic value. If a biopsy sample is to be taken, the testes should be examined ultrasonographically before obtaining the biopsy.[25] The ultrasonographic appearance of the parenchyma can help the clinician to choose a representative site for sampling. Several reports have indicated that obtaining testicular biopsy samples in the stallion can be done safely and with minimal permanent damage to the remaining testicular parenchyma.[26,27] However, many of these studies were performed on normal stallions, and thus the risk to an already compromised testicle (eg, a degenerating testicle) is more difficult to ascertain. Clinicians must carefully weigh the diagnostic benefits of obtaining a biopsy sample against the risk of damaging some portion of an already marginally functional testicular parenchyma.

A final management recommendation is to freeze the semen of valuable stallions when they are at the peak of their fertility. This provides an insurance policy against reproductive loss should a stallion's fertility be compromised by TD or for any other reason in the future. Since the Jockey Club does

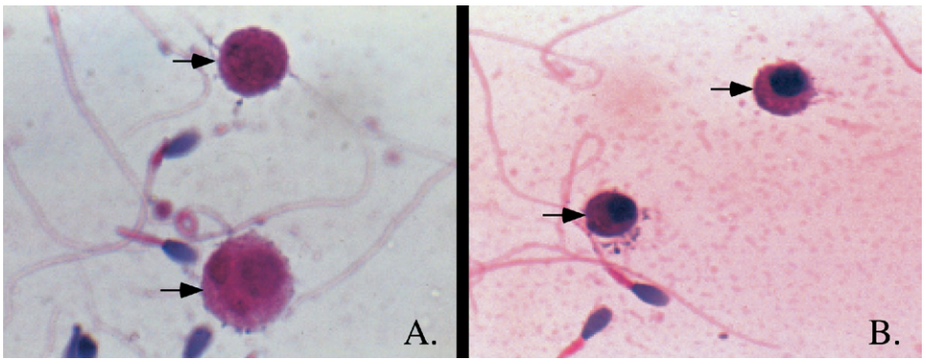

Figure 2 Immature spermatogenic cells (arrows) in stained smears of ejaculates from two stallions with idiopathic testicular degeneration (A, B). Spermatogenic cells vary in size and typically possess round, dark-staining nuclei. This is in contrast to the homogeneous size of white blood cells and the distinctive nuclear characteristics of the neutrophil.

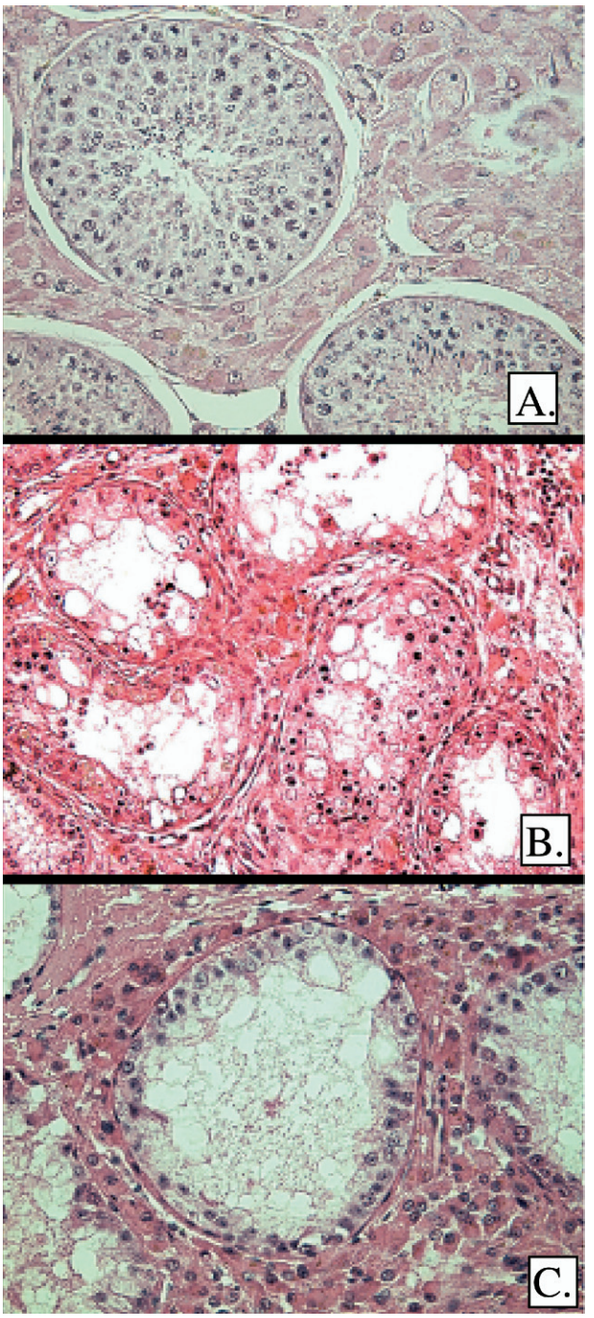

Figure 3 Histologic appearance of the seminiferous epithelium of a fertile stallion (A) and those of stallions with moderate (B) and severe (C) testicular degeneration. In the normal stallion (A), all stages of spermatogenesis are evident, including testicular spermatozoa. The germ cells are arrayed in orderly associations. In the moderately affected stallion (B), immature germ cells are seen being shed prematurely into the seminiferous tubule lumen. There is a loss of the normal architecture of the seminiferous epithelium and an absence of haploid germ cell stages. Additionally, there is vacuolization of the seminiferous epithelium. Other sections from this animal's testes still contained tubules supporting normal spermatogenesis. Although semen quality was poor, the ejaculate still contained low numbers of morphologically normal, progressively motile sperm. In the severely affected stallion (C), there is an apparent absence of germ cells, with the possible exception of a few spermatogonia along the basement membrane. The tubule contains almost exclusively only Sertoli cells. We identified no normal tubules in other sections from this stallion's testes, and the animal was azoospermic.

not currently permit the use of frozen semen, this may not be a viable option for some Thoroughbred sires.

Treatment

There is no known, proven successful treatment for TD. If the cause of the degeneration is known (eg, fever, toxin), successful treatment or removal of the inciting cause should at least prevent further progression of the disease. In acutely affected cases, referral should be considered in the face of uncontrolled pain or swelling of the scrotum, if the condition necessitates placement of an indwelling venous catheter or requires intensive nursing care, if orchiectomy of a diseased testicle is indicated, or in the face of uncontrolled hemorrahge following traumatic injury. If the degeneration is not severe and if the inciting cause is removed, the testicle may at least partially, and sometimes fully, recover depending on the degree of damage sustained.

In cases of unilateral TD, some have recommended removal of the affected testis. The reasoning behind this recommendation is that the damaged testicular tissue could result in the production of antisperm antibodies[28] that might adversely affect sperm produced by the normal testis. Additionally, removal of one testis often results in hypertrophy of the remaining testis and a resultant increase in sperm numbers. The practice of unilateral castration is debatable, however, as there are reports of acceptable fertility in stallions with unilateral TD in which the affected testis was not removed.[1]

There are some reports of the successful use of GnRH therapy as a treatment for infertility in stallions.[29,30] However, these successes have not been duplicated in controlled studies.[12,31,32] Although GnRH therapy has been highly successful in treating men with hypogonadotropic hypogonadism, this condition has not been clearly documented in stallions and our studies strongly suggest that a lack of gonadotropins is not the underlying cause of severe ITD in the horse. In addition, our studies on xenografts of equine testes severely affected by ITD have identified no improvement in the condition of the testes following treatment of host mice with exogenous gonadotropins or provision of the mice with a source of endogenous hormones from normal, functional testis xenografts.[19] If all of this information is taken together, the use of GnRH implants or pulsatile administration of GnRH as a treatment for stallion infertility in general or ITD specifically becomes highly questionable. If this therapy is to be attempted, it has been suggested that treatment must start early, before the testis has reached a severe state of degeneration.[33]

There is support in the literature for beneficial effects of fat-soluble antioxidants (eg, docosahexaenoic acid, DHA) on semen quality in stallions.[34] However, the effects of this treatment on stallions with fertility problems (and specifically with ITD) have not yet been determined to the author's knowledge. Nonetheless, it has been suggested that supplementing marginally fertile stallions with neutriceuticals containing DHA or similar substances (eg, ProSperm; Minitube of America, Verona, WI) might be beneficial to semen quality.

Because there is no proven treatment for TD per se, the basis of dealing with this problem centers around stallion

management. The veterinarian first should determine the number of progressively motile, morphologically normal sperm that the stallion is capable of producing on a regular basis. The stallion's mare book then should be adjusted accordingly to insure that the stallion is not overused. If a stallion with low or marginal sperm numbers is required to breed daily, it is not uncommon for the animal's sperm numbers to drop below what would be required for a minimum insemination dose. Limiting the animal's book so that he has one or more days of sexual rest between each ejaculate often can help boost sperm numbers and improve pregnancy rates in mares. If possible, the semen quality of each ejaculate should be monitored to be certain that each mare is receiving a minimum insemination dose. Addition of an extender to the ejaculate may help improve longevity of sperm motility in some cases.

Semen from stallions with severe TD should be handled with particular care. Mares should be inseminated as quickly as possible after semen collection. Semen should be carefully evaluated as to its suitability for cooled transport. However, in many cases of moderate to severe TD, sperm longevity of motility is poorly maintained and pregnancy rates may be significantly reduced in mares bred with cooled semen. If this is the case, it may be prudent to discontinue the use of shipped semen and only breed mares on site with fresh, extended semen or by natural cover.

More intensive mare management also can be used to improve pregnancy rates. By breeding mares very close to the time of ovulation, and in extreme cases within 6 hours post ovulation, the veterinarian can minimize the requirement for sperm longevity. A final option for management would be the use of assisted reproductive techniques, such as Intracytoplasmic Sperm Injection (ICSI). This technique involves the injection of a single sperm directly into the cytoplasm of a mare's egg and subsequent transfer of the embryo into a recipient mare's uterus. Recent reports indicate ever-increasing pregnancy success following ICSI.[35] Since only one sperm is required for fertilization, ICSI allows for the production of offspring even from severely azoospermic stallions. However, the expense is significant and not all breed registries approve of this technique.

Summary

If a cause for TD can be identified, it should be treated or eliminated. In these cases, depending on the degree of testicular damage, testicular function should improve and possibly return to normal over a period of months.

If a cause for the TD is not known, then by definition the case should be classified as ITD. Changes in plasma hormone levels, particularly during the early stages of ITD, can be variable. As such, some of the most reliable signs of ITD can be identified as part of the routine breeding soundness examination. These include small, soft testes, poor semen quality, low numbers of sperm for testicular size, and the presence of immature germ cells in the ejaculate. Unfortunately, by the time changes in testicular size are noticed, the damage to the testes is already significant. As such, valuable animals should be monitored carefully with regular semen evaluations and testicular measurements to try to identify subtle changes in semen quality and sperm numbers over time. Currently, there is no known successful treatment for ITD. For valuable stallions, and if breed registries permit, semen should be frozen while the animal is at his peak of fertility. This can be banked as an insurance policy in anticipation of the possibility of ITD as the stallion ages. Stallions diagnosed with ITD should be managed intensely to maximize fertility in the face of progressively declining semen quality.

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Infectious Diseases in Breeding Stallions

Kristina G. Lu, VMD, DACT,* and Peter R. Morresey, BVSc, DACT, DACVIM†

A wide variety of infectious bacterial, viral, and protozoal reproductive pathogens have the potential to be transmitted by the breeding stallion. In addition, nonvenereal diseases can also be spread due to the close proximity of mares and stallions during natural service. Disease spread by assisted breeding techniques is also possible. The equine veterinarian must develop a thorough understanding of the relevant diseases and be able to formulate treatment and management plans to minimize the effects of infection on breeding programs.
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The equine veterinarian plays a vital role in monitoring for and preventing sexually transmitted diseases. Natural cover allows the spread of venereal disease via direct mucosal contact, as well as permitting dissemination of other diseases that may spread via respiratory secretions. Sexually transmitted diseases are not limited to natural breeding, as artificial insemination is permitted by a vast number of breed registries, removing the requirement for proximity of mares and stallions. The ability to transport equine semen over large distances and to preserve genetics over time has led to many breed and economic benefits. However, widespread dissemination of sexually transmitted diseases is also possible without strict control of breeding practices and laboratory methods.[1]

Bacteria and Mycoplasmas

Specific bacterial venereal infections do occur. Pathogenic organisms such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Streptococcus zooepidemicus* may cause significant infertility in situations where they are permitted to colonize and multiply. The commensal bacterial flora of the external genitalia is not regarded as pathogenic, and in fact performs a useful role in increasing resistance to superinfection with the above detrimental organisms. Although present in the ejaculate, commensal organisms have not been shown to diminish reproductive performance (Figs. 1-3).

Contagious Equine Metritis

Contagious equine metritis (CEM) is a highly transmissible bacterial infection caused by *Taylorella equigenitalis*, a fastidious, microaerophilic, Gram-negative coccobacillus. *Taylorella equigenitalis* is typically sensitive in vitro to multiple antibiotics; however, the organism is divided into two biotypes based on sensitivity or resistance to streptomycin.[2] Clinical signs of CEM are limited to the mare reproductive tract and can vary from overt to subclinical disease. The incubation period is 2 to 12 days. Clinical signs include odorless, gray to white, mucopurulent vulvar discharge associated with endometritis, cervicitis, and vaginitis that result in temporary infertility. Re-exposure is associated with minimal to no clinical signs.[3,4] Abortion in pregnant mares is rare.[4,5]

Taylorella equigenitalis is venereally transmitted by asymptomatic carrier stallions or by infected mares during intromission, and inapparent carrier mares or stallions are responsible for spread of disease. Additionally, the bacteria may be spread by fomites. Transplacental transmission has been reported resulting in congenital infection, contamination, or abortion.[4-6]

A treatment protocol for the stallion external genitalia includes 5 consecutive days of cleaning with 4% chlorhexidine gluconate and application of an antibacterial ointment such as 0.2% nitrofurazone.[7,8] An alternative topical treatment is 1% silver sulfadiazine cream mixed with 10 mL Quartermaster (Pharmacia and Upjohn), a bovine intramammary treatment.[7] Systemic antimicrobial treatment may be an important adjunct to topical therapy. Systemic antimicrobials described for CEM treatment include procaine penicllin and trimethoprim sulfamethoxazole (30 mg/kg PO BID).[7,9]

Effective treatment of *T. equigenitalis*-infected mares is challenging since there is some evidence that treatment may lead to persistence of the organism in the clitoris.[3,4] Treatment of the clitoris includes 5 days of smegma removal, irrigation with 4% chlorhexidine, and packing with 0.2% nitrofurazone or similar ointment. Ultimately, removal of clitoral tissue may be necessary for resolution of the carrier state.[3]

Testing for *T. equigenitalis* originates with obtaining swabs

*Hagyard Equine Medical Institute, Lexington, KY.
†Rood and Riddle Equine Hospital, Lexington, KY.
Address reprint requests to: Kristina G. Lu, Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511. E-mail: luk@alumni.upenn.edu

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Figure 1 Sampling penile shaft for microbial culture. (Color version of figure is available online.)

Figure 3 Sampling urethra for microbial culture. (Color version of figure is available online.)

of potentially contaminated sites. In the mare, these include the clitoral sinuses, the clitoral fossa, and the endometrium in the nonpregnant mare. In the stallion, sites for swabbing include the urethral sinus, the penile shaft and prepuce, and the fossa glandis. Swabs are placed in Amies medium supplemented with charcoal. Traditionally, swabs are used for microbial culture followed by identification of the organism by morphological and biochemical methods. Drawbacks of this method include the time required for microbial culture and overgrowth of contaminant bacteria or fungi. An alternative method of diagnosis utilizes the polymerase chain reaction (PCR), which has been reported to be more sensitive than bacterial isolation for detection of *T. equigenitalis* from genital swabs. Accuracy of PCR results may be enhanced by a culture step.[10] Additionally, a PCR method has recently been validated to test directly from swabs.[11]

Control is predominantly through testing of imported horses to prevent spread of disease. Even with comprehensive standardized testing protocols, potential for transmission has been demonstrated to occur.[7]

Taylorella asinigenitalis, an organism closely resembling *T. equigenitalis*, has been isolated from American donkey jacks. This organism has also been isolated from the urethral fossa, urethra, and penile sheath of a 3-year-old Ardennes stallion during routine CEM testing.[12] A discriminatory real time PCR for *T. equigenitalis* and *T. asinigenitalis* has been developed for the direct examination of genital swabs.[11]

Figure 2 Sampling urethral fossa for microbial culture. (Color version of figure is available online.)

Pseudomonas aeruginosa and *Klebsiella pneumoniae*

The stallion can be an asymptomatic penile carrier of pathogenic organisms that are readily transferable to the mare; however, there is most often no association between the colonizing organism of the stallion and the uterine invader of the mare.[13] *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have an association with endometritis in the mare, especially in those cases where a defect in uterine clearance exists. Factors predisposing stallion genitalia to colonization are not known; however, washing and disinfection may remove commensal flora allowing superinfection.[13,14] Variation in virulence may also be the result of strain variation.

Regardless of the method employed, cleansing of the stallion's penis has been shown to alter the bacterial flora. Soaps favor the growth of coliforms, and iodine encourages growth of both *P. aeruginosa* and *K. pneumoniae*.[14] Dilute hydrochloric acid (0.1%) is considered to be less disruptive and can eliminate *P. aeruginosa*.[13] The use of a 0.5% sodium hypochlorite solution, conveniently made with household bleach, has also been reported to eliminate *K. pneumoniae* (RM Kenney, personal communication).

Mycoplasmas

Mycoplasmas have been implicated in reproductive dysfunction of horses. Mycoplasmas are present as commensals in the genital tract of otherwise healthy stallions. In one study, they were isolated predominantly from the fossa glandis and urethra and less frequently from the penis shaft and from semen.[15] This raises the possibility that clinically healthy stallions may present a permanent reservoir of infection for mares via venereal transmission.

Viral Infections

Venereal transmission of certain viral infections can occur. The ability of the Equine Arteritis virus (EAV) to be transmit-

ted by semen and cause reproductive failure in susceptible mares is well established. Equine infectious anemia (EIA) virus has been found in semen from infected stallions; however, there is no evidence that transmission of disease has occurred. Equine herpes virus type 3 (EHV-3), a highly contagious virus, has also shown the ability to be transported in semen and cause equine coital exanthema, a condition manifest as painful lesions on the stallion's penis and mare's vulva. Fomite spread of EHV-3 also occurs.

Equine Viral Arteritis

Equine viral arteritis (EVA) has recently received increased attention because of the 2006 outbreak in the American Southwest associated with cooled transported semen. This increased attention and timely response is encouraging in the face of the USDA's National Animal Health Monitoring System Equine 1998 study, which found that 58.4% of horse operation personnel or owners had never heard of EVA, 27.6% were familiar with the name but had little knowledge, and 13.0% were aware of at least basic information regarding EVA. The NAHMS 1998 study found that 2.0% of unvaccinated horses were EVA-positive. Standardbreds had the highest seropositive percent at 23.9%.[16] By way of comparison, a 2005 Hungarian study reported a seroprevalence in that country of 65%.[17] Serological investigations indicate that EAV has a worldwide distribution and that its prevalence is increasing.[18]

EAV is an enveloped RNA virus in the family *Arterivirdae*. Other arteriviruses in veterinary medicine include porcine reproductive and respiratory syndrome virus, simian hemorrhagic fever virus, and lactate deydrogenase-elevating virus in mice. The virus replicates in endothelial cells and macrophages and subsequently spreads to regional lymph nodes. The virus also targets some epithelial cells, mesothelium and smooth muscle cells of arteries, as well as the myometrium. Whereas the majority of EVA infections are asymptomatic, clinical signs of EVA are primarily associated with endothelial cell injury and vasculitis, including fever, malaise, serous or mucoid nasal discharge, and edema of limbs and dependent areas, including the sheath, conjunctivitis, stomatitis, and urticaria.[19] Pregnancy loss or abortion may occur with rates reported from 10% to 60%.[20] Interstitial pneumonia has been observed in newborns.[21]

Transmission of EVA may occur through multiple routes with respiratory transmission most common. Spread can also occur through venereal transmission via semen (fresh, cooled, and frozen), contact with an aborted fetus or placenta, and potentially urine, feces, and vaginal fluid. Viral incubation requires approximately 3 to 14 days and respiratory shedding occurs for 7 to 14 days. In challenge models, there appears to be no appreciable shedding from various tissues after 28 days postinfection.[19,22] Bedding or other fomites contaminated with EVA- infected semen can allow lateral transmission from shedding stallions to susceptible in-contact horses.[23]

After natural EVA infection, most horses develop long-term immunity to the disease. A testosterone dependent, asymptomatic carrier state exists in 30% to 60% of infected stallions. The virus resides in the ampulla or vas deferens and is associated with constant shedding of the virus. In surveys performed by the Gluck Equine Research Center, EVA carrier stallions have been detected in a wide range of equine breeds ranging from 22% in nonvaccinated Thoroughbred stallions to 55% in Standardbred stallions. The carrier state has not been detected in a seronegative stallion or in a stallion vaccinated with the modified live virus vaccine (ARVAC; Fort Dodge Animal Health).[24]

EAV is an RNA virus and is thus associated with high mutation rates and development of variants, or quasispecies, during persistent infection in carrier stallions. This virus evolution in carrier stallions may change properties of EVA such as its ability to resist antibody neutralization and its virulence.[25]

Treatment of the affected animal consists of supportive care and management of any secondary bacterial infections. The virus is generally eliminated from the tissues of an infected horse by 28 days after infection, and this is correlated with the appearance of neutralizing antibodies. Neutralizing antibodies are persistent and associated with long-term immunity to reinfection.[22,26,27] Foals born to immune mares are protected from clinical EVA infection by passive transfer of colostral antibodies. Colostrum derived antibodies wane between 2 and 6 months of age.[26,28,29] Studies suggest that colts exposed to EVA before puberty establish immunity without becoming carriers.[30]

Castration is a treatment option for the chronic EVA carrier state in stallions.[31] Attempts have been made to use GnRH antagonists to temporarily cease EVA shedding in stallions.[32] Treatment of semen has been described to remove the virus. This treatment includes a combination of density gradient centrifugation and a "swim up" procedure. The techniques described were borrowed from human literature describing removal of infectious human viruses before use in assisted reproductive techniques.[33]

Diagnostic tests for EVA are indicated when an equid presents with suggestive clinical signs, for stallions before entry into a breeding program, or as part of routine surveillance. Diagnosis of EVA can be made with a four-fold increase in serum antibody titer, comparing antibody levels between acute onset of clinical signs and again 3 to 4 weeks later. Virus may be identified from whole blood or nasopharyngeal secretions in acute infections. Virus identification is performed by virus isolation or by reverse transcriptase-polymerase chain reaction (RT-PCR) methods. Heparinized blood samples should not be used because of the inhibitory effect of heparin on the isolation of the virus in cell culture.[19,34] In carrier stallions with a titer greater than 1:4, semen is evaluated for presence of EVA virus by virus isolation or RT-PCR methods.[19] The ideal sample is the sperm-rich fraction of an ejaculate containing at least two billion sperm.[35] In a study of EVA-associated abortion, PCR and immunohistochemistry were found to be the most sensitive and useful diagnostic tests for diagnosis of EVA abortion.[17]

Control of EVA is established by isolating suspicious clinical cases to prevent aerosol spread of disease, by serological testing of stallions to detect asymptomatic carriers, and by vaccinating horses at risk of exposure. Documented shedding stallions should be bred only to seropositive mares, whether by natural infection or as the result of vaccination. These mares should be isolated from seronegative horses for at least 3 weeks after breeding.[18]

The Animal and Plant Health Inspection Service (APHIS) recommends that all EVA-negative colts under 270 days should be vaccinated. Additionally, all breeding stallions should be tested and imported semen should be evaluated before breeding. APHIS guidelines suggest that a mare to be bred to a carrier stallion should be isolated for 21 days after breeding due to potential for virus shedding (http://www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/fs_ahequineva.pdf). Documentation of serologically negative status before vaccination is critical. International travel may be restricted with EAV-positive serology, particularly without prevaccination serology documentation.

Equine Herpesvirus 3

Equine coital exanthema is caused by Equine Herpesvirus 3 (EHV-3). Superficial pock-like erosions or ulcers occur following venereal transmission. While being highly contagious with post coital infection rates as high as 100%, EHV-3 is noninvasive and benign. Clinically apparent equine coital exanthema (ECE) occurs only sporadically in most breeding establishments, and the overall incidence is unknown. Systemic illness, infertility, and abortion do not occur naturally, although abortion has been induced experimentally.[36]

Virus reactivation from latency with recrudescence of infection serves as the source for spread of EHV-3. The anatomical site that harbors the latent herpesvirus is unknown. The primary method of virus transmission is through direct skin-to-skin contact by coitus with an actively infected, virus-shedding horse. Viral shedding is intermittent, with continuous shedding not yet identified. The incubation period is 5 to 9 days. Evidence exists that subclinically infected horses without visibly noticeable lesions can also transmit the virus to their breeding partners.[37]

Outbreaks of EHV-3 may result from a mare brought to the stud farm for breeding, or reactivation from a member of the resident stallion or mare population. Iatrogenic transmission of infection can occur by virus-contaminated objects used for breeding, grooming, palpation per rectum, or gynecological examination. Virus spread during artificial insemination has not been investigated, and noncoital transmission by genital to nasal contact may be possible. Mechanical transmission by stable flies has also been suggested.

Figure 4 Healing ulcers caused by EHV-3. (Color version of figure is available online.)

Figure 5 Healing ulcers caused by EHV-3. (Color version of figure is available online.)

Infection with EHV-3 results in an intense localized inflammatory response, with systemic spread not reported. Individual variation in severity occurs. The resulting lesions are characteristic, progressing from a small papule, to a vesicle, pustule, and finally shallow crusting erosions over the space of a few days. Edema of the prepuce may occur, extending laterally onto the ventral abdominal wall and scrotum. Secondary bacterial infection with *Streptococcus equi* ss *zooepidemicus* is common, influencing the severity and duration of lesions. Lesions in varying stages of progression can occur concurrently. Recovery is complete in 2 to 3 weeks (Figs. 4 and 5).

Diagnosis is usually made by observation of the characteristic genital lesions. To facilitate the discovery of smaller lesions potentially hidden in penile or preputial folds, the penis can be reliably extruded by the use of xylazine. Confirmation of infection can be attempted by PCR, by isolation of the virus, or by demonstration of either seroconversion or a four-fold rise in antibody titer in paired serum samples taken 3 to 4 weeks apart. Antibody response to infection may be minimal, however this does not indicate a lack of viral challenge.[37] Further specimens for laboratory confirmation include material collected from the edges of fresh, active lesions.

The overall therapeutic objective of the treatment of breeding stallions exhibiting coital exanthema is promoting the rapid and uncomplicated healing of genital lesions so as to shorten the period of sexual rest. Daily cleansing of the genitalia, reduction of inflammation, and prevention of secondary bacterial infection are the principles of treatment. Therapy is palliative and prophylactic rather than curative. In uncomplicated cases, healing of lesions is complete by 10 to 14 days, however persistent depigmented scars remain.

The impact of EHV-3 on equine breeding enterprises is primarily due to temporary cessation of breeding activity. This may lead to significant decreases in the number of mares covered by affected stallions. Reproductive efficiency is further decreased by delay in foaling dates and reduced pregnancy rates as a result of mares missing opportunities to breed.

As reactivation of latent virus is not preventable, the basis

for controlling the impact of outbreaks of ECE in breeding establishments is containment of the spread of infection. A stringent code of practice should be implemented within breeding sheds following observation of a case of ECE. The three priorities necessary for successful ECE control are:

- Cessation of breeding of clinically affected animals.
- Heightened vigilance on the part of personnel for early recognition of new clinical cases.
- Strict adherence to breeding shed hygiene procedures designed to eliminate mechanical transmission of the virus.

The decision to return an EHV-3-infected stallion to breeding service should be based on a clinical evaluation of genital lesions rather than on a fixed length of time. Withdrawal period will be influenced by the extent and severity of the lesions and by the rapidity of the healing process. Regression and granulation of lesions makes the likelihood of continued viral shedding low. The recovery period will be prolonged by secondary bacterial infection.

Protozoa

Dourine

Dourine is a disease confined to *Equidae*, with natural infections reported only in horses and donkeys. The causative agent is classically thought to be *Trypanosoma equiperdum*; however, overlap has been noted between this species, *T. evansi*, and *T. brucei brucei*.[38] Only a small number of laboratory strains of uncertain origin exist from the early 20th century. No recent isolates have been obtained.[39] The disease is found only in Africa, South and Central America, and the Middle East.

Dourine is suspected to only be transmitted by venereal spread. Transmission is not certain, even from matings with known-infected animals. Males may serve as noninfected mechanical carriers after breeding of infected females. Spread by vector seems unlikely as the low number of organisms in peripheral blood of infected horses makes biting insect transmission unlikely. The organism has a tropism for genital mucosa and is thought to be unable to survive outside the host. Risk factors for infection include contact with horses from known affected areas.

Clinical signs are considered to be pathognomonic for the disease. Acute disease following the 1-week to 3-month incubation period is characterized by pyrexia, debility, and multi-systemic disease. Three phases of disease are recognized. Initially, genital edema and tumefaction occur. This includes edema of prepuce, urethral process, penis, testes, and scrotum. Paraphimosis may occur, as can inguinal lymph node enlargement. A purulent urethral discharge is present; however, the organism can be periodically unrecoverable from the urethra. This is followed by the characteristic widespread occurrence of raised cutaneous plaques 1 to 10 cm in diameter which may depigment. Finally, anemia, neurological compromise (hindquarter weakness; ataxia, hyperesthesia, and hyperalgia), emaciation, and death ensues. Severity of clinical signs is dependent on the strain of the organism and the general health of the affected horse population. Approximately 50% of affected animals die of acute disease within 6 to 8 weeks.

Diagnosis is based on the occurrence of characteristic clinical signs and laboratory methods. Cytology may detect the causative organism in smears of body fluids or lymph node aspirates. Seminal fluid and preputial mucus may also yield organisims. Necropsy findings include emaciation with enlargement of lymph nodes, spleen and liver with periportal infiltrations in the liver, and petechial hemorrhages in the kidney. In the nervous form, the organism can be recovered from the lumbar and sacral spinal cord, sciatic and obturator nerves, and cerebrospinal fluid.

No definitive diagnosis is possible at the serological or molecular level, as neither serological nor DNA-based tests can provide subspecies Trypanozoon identification. The complement fixation (CF) test is the most widely used and the only internationally recognized test. However, this test was developed in 1915 and may not accurately reflect current infective strains. The use of AGID, IFA, and ELISA tests has also been reported.

Treatment may be successful if instituted early in the course of disease; however, international regulations impose slaughter on CF-positive horses.

Control is hampered by the presence of asymptomatic carriers. Where the disease occurs, movement of horses from infected areas should be prohibited and breeding should be controlled. Eradication schemes involve serological testing, with slaughter of infected animals. Consecutive negative CF tests at least 1 month apart indicate freedom from disease.

Other Protozoal Diseases

Piroplasmosis, a disease caused by *Babesia equi* or by a less severe strain, *B. caballi*, has increased in importance in recent years due to the increased movement of horses between countries. Found throughout the world, it is considered to be enzootic in many areas of the southern United States. Ticks are considered to be the vector; however, mechanical transmission has been documented. The possibility of venereal transmission via contamination of semen with infected horse blood has also been theorized.[1]

Nonreproductive Disease Transmission in the Breeding Shed

Breeding sheds have the potential to act as a point source for many infectious outbreaks due to the high number of horses transiting these areas in commercial operations. Droplet spread of respiratory pathogens (EHV-1, EHV-4) via direct and indirect contact between horses therefore has the potential to be widely disseminated. Similarly, cutaneous pathogens (dermatophytes) also have the ability to be transferred between stallions and mares.

In the Thoroughbred industry, Jockey Club registration requires natural breeding. Jockey Club records indicate that, during the 2006 breeding season, 3054 Thoroughbred stallions bred 59,444 mares via natural cover. This is merely an example of the potential for various contagious diseases to achieve aerosol and venereal spread and why the equine veterinarian must be vigilant to ensure industry success.

Summary

The stallion can act as a point source of infection for a number of venereal and nonvenereal diseases. The role of the

equine veterinarian, via a thorough understanding of the disease processes, is to maintain vigilance for signs of infection, provide timely and efficacious treatments, and formulate effective breeding management systems to minimize the occurrence of infection, and should it occur, eliminate potential for spread of that infection to in-contact animals.

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Thoughts on Standing Stallions for Natural Service

Walter W. Zent, DVM

Standing stallions for natural service is a commitment that should not be entered into without careful thought and planning. It can be a very exciting and financially rewarding endeavor, but without proper thought, it is apt to turn into a disaster. Proper facilities, adequate competent staff, and well-managed horses are a must if the operation is to be successful. The lack of planning and preparation has the potential to turn a worthwhile idea into a nightmare that will likely deliver poor results and could become a liability to the industry. Good stallion management and disease control are a must for even the smallest of operations. Proper planning and competent advice will be time and money well spent no matter what the size of the operation that is planned.
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Facilities

The breeding shed is the center of activity during the servicing season. It needs to have easy access for both stallions and mares; it should be located so that visiting mares can be accommodated without having to travel through the farm and the resident mares can have ready access with, depending on the size of the farm, as little vanning as possible. If the farm is going to breed a large number of walk-in mares, as is the case in most places here in Kentucky, then thought must be given to traffic flow, unloading areas, and ample area for uncongested mare movement. The unloading area needs to be designed to handle both truck and trailer traffic with both well-designed loading chutes for trucks and an area for trailers to move.

The breeding shed needs to be of ample size so that people and horses can have room to move out of the way of trouble. It needs good footing that is dust-free and will not become slippery when wet. There are many new materials that have been developed that are an improvement on the tanbark and wood chips that have been used in the past (Fig. 1). The designer of a breeding shed would be well served to research the selection of footing before installation, as uneducated choices can prove to be costly mistakes on this most important part of the shed. What looks pretty may not be the most functional.

Breeding sheds need hot and cold water, a stock to wash mares, and a place to rinse the stallion. A clean room that could be used as a laboratory and storage is a handy thing to have incorporated in the shed. If artificial insemination is not being done, this need not be a very fancy place. A bench for a microscope, a small incubator, a small refrigerator, and a sink would be the essentials. All of this can be very simple; the emphasis should be on adequate uncluttered space and good footing. Weather permitting, the best place might be a grassy paddock.

The stallion barn needs to be safe and well designed. Most stallion stalls are at least 14 × 14 feet, as the stallion usually spends more time in the stall than other animals on the farm. Many farms have stallion stalls that are much larger but this probably is not necessary. Ventilation, as in any other barn, is important, and the stallion stalls should be as airy as possible. The stalls should be designed with the doors in the center rather than up against a wall. Horses are much easier to catch if they do not have a long wall between the door and corner. An additional outside door can also be useful, particularly with difficult stallions as two people can approach them from different directions. An outside door will also add ventilation and could act as an additional exit in case of fire.

Paddocks should be of good size, well fenced, and provide some shade if possible. It is useful if the entire paddock can be viewed from the gate so that animals can be located before entering the paddock. This not only makes observation easier but also reduces the likelihood of two stallions accidentally being turned out in the same paddock. The location of the paddocks should be as close as possible to the barn for ease of handling and safety. The less distance that the animal must be led, the less likely he is to get loose.

Personnel

The number of personnel that are needed for a stallion operation certainly depends on the number of stallions that are being bred. A minimum would be three; none of these needs to be dedicated to the stallion operation if the farm is small,

Hagyard Equine Medical Institute, Lexington, KY.
Address reprint requests to Walter W. Zent, Hagyard Equine Medical Institute, 4250 Iron Works Pike, Lexington, KY 40511. E-mail: wzent@hagyard.com

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Figure 1 The breeding shed should be spacious and free of obstructions. Synthetic flooring materials offer good secure traction and drain well. A heavily padded buttress can be used to help keep the mare from moving forward when mounted. (Color version of figure is available online.)

but it certainly makes for a better operation if there is a team that is used to working together and they have jobs that they are used to doing. One person should handle the stallion and two people for the mare: one for the head and one for the tail (Fig. 2). A farm that has walk-in mares will be better off to have their own help and not depend on the person who comes with the mare for help. They are often not good horsemen; they may be only a van driver, and incompetent help is the biggest danger in the shed. Remember that usually everything goes fine, but when things go wrong they can go wrong in a hurry. Having people that are used to reacting can often prevent a serious incident from occurring.

A very important person in any operation that breeds any number of outside mares is the booking secretary. This person schedules the breeding of the mares, and for a stallion that has a large book, they are an extremely important part of any operation. Central Kentucky is blessed with a very talented office staff whose job it is to book the covering of several stallions. When the mares are coming from literally scores of different farms and the stallions are breeding over 100 mares, this is a huge job. The booking secretary needs to be thick-skinned with a pleasant personality and a good sense of humor. It is a very demanding job keeping both the mare owner and the stallion manager happy.

Mare Management

Stallion management must be based on good mare management. Paying attention to a stallion's book (the mares scheduled to be bred) can go a long way to increasing the stallion's performance. Mares should be selected with some regard to when they may arrive at the shed. If 50% of a stallion's book was made up of April foaling mares, it could make for an active May. This would limit the number of mares the stallion could breed because there simply are not enough days to get them covered. On the other hand, if the horse has mares coming to him from the 15th of February forward, he will have a much easier time of it. Stallions that have a large percentage of their book residing on the farm where they stand are certainly at an advantage over the stallion that has mostly outside mares. The resident farm mares can be examined and a decision on whom to breed can be made at the last minute, thus conceivably limiting the number of covers that must be made. Care must be taken, however, not to breed mares just because the stallion is there. Remember, the act of breeding mares can be dangerous, and serious breeding shed accidents do occur, so indiscriminate covering of mares is ill advised. The mares should be checked and the best candidates bred at the appropriate time. Close monitoring of the farm mare will also allow the stallion manager to move the covering of a farm mare to allow for the breeding of a walk-in mare that is closer to ovulation. Remember that the object of stallion management is to get the most mares in foal on the least amount of covers.

Booking the nonresident, walk-in mare can be a little more difficult because the booking secretary and the stallion manager do not have direct control of the mare and must take the word of an outside mare manager. This requires trust and team effort. If everyone works together, it can be very successful. Good booking secretaries soon learn the players, and have a good idea of the abilities of the outside veterinarian and farm manager. They will work hard to get the mares in the proper spots when they are getting good information and cooperation from all the parties.

When the walk-in mare arrives at the breeding shed, everyone should remember that this is a strange place for her and she may act differently than when she was at home. The teaser is strange. She doesn't have her foal. She doesn't know any of the other animals around her. The mare may become threatened and act accordingly. The handlers in the breeding shed should also remember that the mare is a potential spreader of disease, and it is wise to keep nose-to-nose exposure to other horses to a minimum. Most people would not intentionally send a sick horse to the breeding shed, but it has happened and the results can be devastating.

The use of the teaser at the breeding shed is important to protect both the stallion and mare from injury (Fig. 3). In

Figure 2 Many farms will have a stallion handler and two mare handlers. One mare handler will hold the mare's head and apply a twitch, and the other mare handler will hold the mare's tail to the side. (Color version of figure is available online.)

Figure 3 The teaser stallion should wear a well-adjusted stout halter with large cheek rings that allow the chain of the lead shank to slide easily. Teasers that test mount mares should be fitted with a shroud to prevent accidental intromission. (Color version of figure is available online.)

central Kentucky, almost all mares are teased at the breeding shed. A mare's reaction to the teaser will govern how much restraint, both manual and chemical, is used or whether the mare is bred at all. Some mares will show better at the breeding shed than they do at home, and some will show less. The teasing should be done as completely as need be to judge how the mare will react to the stallion. Good communication between the farm manager of the mare and the stallion manager can be very helpful and give the mare a better chance of getting bred.

The aggressive management of mares is the most important contribution to the management of a stallion's book. The Thoroughbred breeding season is short enough that mares must be managed aggressively for everyone to get a chance to be covered by busy stallions. The use of artificial lighting programs and other estrus management systems are a great benefit not only to mare management but also to the management of the stallion. The use of artificial lighting to hasten the period of transitional estrus and allow for most of the transition to occur before the start of the breeding season has been a great help to stallion managers. When mares are acting properly at the beginning of the breeding season, a larger percentage of the maiden and barren mares will be bred before the foaling mares begin to arrive in earnest. Programs for estrus synchronization are also very helpful for both on-farm and walk-on mares. Some of these programs work so well that mares can be booked several days before they come into heat, and the stallion manager will know when to expect outside mares to their stallions. The breeding of mares can also be spread out so that a stallion with a large group of barren and maiden mares in his book will not receive all of them on the same day. Along with estrus synchronization, the use of ovulation-enhancing drugs will also help to reduce the number of covers needed and improve conception rates by allowing mares to be bred closer to ovulation without the necessity of return covers.

Stallions

The stallion needs to be prepared for the breeding season as well. The young stallion needs to be schooled to breed mares in hand in the breeding shed. A test mare should be chosen that is quiet and reproductively sound. The mare should be bred when in good heat, and an effort should be made to teach the horse good habits. This can be time-consuming, and the stallion may still develop habits that can make him difficult to breed. The time taken in the beginning of a stallion's training can greatly reduce bad habits. Many stallion managers will breed all of their stallions a few times before the start of the season. During this period, it is often wise to do a breeding soundness examination to make sure that nothing has changed since the end of the season that may require adjustment in the stallion's management. This should be done on all stallions unless otherwise indicated. Some stallions that have no experience using an artificial vagina may be difficult to collect, and often it might be better to leave them alone if no problem is suspected, rather than giving them a bad experience in the shed. Semen can be collected from most stallions by an accomplished operator with patience.

Once the breeding shed is open, the preparation of the stallion for breeding is important but in some cases very overdone. Excessive washing of the stallion's penis has proven to be contraindicated. Rinsing with clear water before and after breeding will not be detrimental and is all that is needed in most cases. Excessive washing with antiseptics has been shown to increase the presence of pathogenic bacteria. Routine culturing of stallions is practiced in many breeding sheds. It does no harm unless overinterpreted. There is very little indication for treating a stallion's penis on the basis of a bacterial culture unless he is actually infecting mares. More harm is done overtreating stallions on the basis of a culture than leaving them alone. Having said that, aerobic culture of swabs of the stallion's genital tract that produce a heavy growth of a pathogenic organism, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and β-hemolytic *Streptococcus sp*., should be evaluated and the mares that he breeds monitored for uterine infections.

The frequency with which a stallion covers mares is determined by many things, the most important being his popularity. If no one has mares they want to breed to him, the stallion will not cover many mares. After that, his health, age, libido, and fertility will be the determining factors. A stallion's fertility will determine how many covers are made per conception, and as this number goes higher, of course the number of mares he will be able to successfully breed will go down. The number of covers that normal fertile stallions can make per day is much higher than we thought years ago. At the height of the breeding season in Kentucky, most stallions are able to breed three times a day for several days without a decline in fertility. Less fertile stallions may need to breed less often to give them time to replenish their sperm numbers. Older horses that have testicular degeneration may need a day or two to rebuild their sperm numbers, but will get mares in foal if given time to replenish. Making these management decisions is the job of the stallion manager and veterinarian after review of the breeding records and conception rates. Judicious management of stallions and making the difficult

Figure 4 Mares are often fitted with a protective neck shroud that has sturdy flaps that can be grasped by the mounted stallion for support. The mare's tail is wrapped to prevent injury to the stallion's penis. Restraint includes a halter, long lead shank, long handled twitch, and a front leg strap. Hind feet are sometimes fitted with heavily padded breeding boots. The mare is positioned on a large cocoa mat in front of the heavily padded buttress. Note the helmet, body protector, and gloves worn by the mare handler. (Color version of figure is available online.)

decision to reduce a subfertile stallion's book will result in more total mares becoming pregnant. These types of management changes can be difficult, particularly in the middle of the breeding season, but by monitoring stallion performance, they can be made with the end result being an improvement in overall pregnancy rates.

Not all mares that are presented to the breeding shed are in perfect standing heat. Some are not in estrus because they have been poorly evaluated by their attending veterinarian and/or farm manager, some are not showing because they have been given drugs of some sort to try and prevent them from showing estrus when they have been performing or racing, and some just psychologically are not going to show. In the Thoroughbred world, where natural cover is the only method permitted, these mares can be a challenge. Great effort is usually made to prevent both the mare and stallion from getting injured (Fig. 4).

The mare that has been incorrectly evaluated will usually show better when properly prepared, and usually a conversation with the farm manager can solve the problem. Mares that have been given anabolic steroids, long-acting progesterone, or other drugs to prevent signs of estrus can be a problem when the mare returns from the training facility to be bred. In many cases, the farm will simply have to wait until the drug is out of her system. This can take a few days to months, depending on what drugs have been used. The mare that psychologically will not show is another problem, and steps must be taken to try and get her covered.

Mares are often protective of their foals and will tease better when the foal is not nearby. It is also not unusual for mares to not show at home and then be in good estrus in the environment of the breeding shed. If the breeding shed staff will take some time and patience with mares that do not show well, they can usually get them bred. It is important for manager of the farm of origin to communicate with the breeding shed staff so that they are aware of the mare's nature. It is also the farm's responsibility to make sure the mare is as physiologically right as they can get her. If sedation is needed, it is usually best to administer it before the mare becomes too excited. Sedate the mare before you get in a fight, not after. Mares that will not tolerate the teaser's advances and have been bred earlier in the breeding season should be examined to ascertain that they are not already pregnant.

Having said the above and with all the best efforts, if a mare does not show and the teaser can't mount her, even with chemical restrain, it is the responsibility of the stallion manager to send the mare home. The safety of the farm help, the stallion, and the mare are his responsibility, and if he is not comfortable trying to breed the mare, it his duty to stop any further breeding attempts. Stallions, mares, and staff have all been severely injured and even killed in the shed trying to force the issue.

Miscellaneous Thoughts

In conclusion, it is important to remember that the breeding shed can be an important place for the dissemination of disease. In my 40 years of practice in central Kentucky, I have seen several instances where disease conditions have been disseminated through the breeding shed. A2 influenza in 1964 was brought to a naive population of stallions from the race track and spread across the Bluegrass from the breeding shed. In 1978, Contagious Equine Metritis was imported with stallions from France and again spread around the area disrupting the breeding season.[1] Equine Viral Arteritis in the 1980s was brought to a breeding shed from the race track and again disseminated across the area. Breeding shed managers must always be aware of these types of threats and aggressively investigate any condition that arises in the stallion band. Unfortunately walk-in mares are a necessity to most successful stallion operations, but they also pose a health threat and must be continually monitored. The mare manager also has the responsibility not to send a mare that may have been exposed to an infectious disease to the breeding shed, as this can be a threat to the entire industry in any given area.

From a public relations standpoint, it is important for the stallion manager to remember that the breeding shed is the most visible place in the horse breeding community and the operation should be presented well as the entire farm will be judged on how the shed looks and is run. The same is true of the farm sending mares; they will often times be seen by more people at the shed than anywhere else, and their whole operation will be judged by how well these mares look.

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